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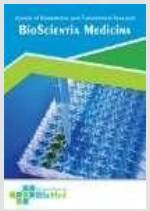

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Biallelic versus Monoallelic TP53 Inactivation in Hematologic Malignancies: A Comparative Meta-Analysis of Lymphoid and Plasma Cell Disorders

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A B S T R A C T

Background: The tumor suppressor protein p53, encoded by the TP53 gene on chromosome 17p13.1, functions as the central guardian of genomic stability. In hematologic malignancies, including multiple myeloma (MM), chronic lymphocytic leukemia (CLL), and myelodysplastic syndromes (MDS), p53 dysfunction acts as a universal marker of chemoresistance and disease progression. Current clinical staging systems frequently conflate chromosomal deletion, or del(17p), with somatic mutation, thereby failing to distinguish between monoallelic (single-hit) and biallelic (double-hit) inactivation. This lack of granularity obscures the distinct biological consequences of these two states. This study aims to resolve the prognostic discordance between genomic subgroups in the context of modern therapeutic interventions. **Methods:** We conducted a comparative meta-analysis of nine pivotal studies comprising 4,125 patients, selected through a stringent protocol requiring paired cytogenetic (FISH) and molecular sequencing data. Patients were stratified into three genomic subgroups: Wild type, monoallelic disruption (isolated deletion or isolated mutation), and biallelic disruption (deletion plus mutation). Data were synthesized using a random-effects model to calculate pooled hazard ratios (HR) for Overall Survival (OS), with specific subgroup analyses performed for Plasma Cell versus Lymphoid malignancies to account for lineage heterogeneity. **Results:** The analysis revealed a profound prognostic dichotomy. In Multiple Myeloma, biallelic inactivation conferred a catastrophic prognosis with a pooled hazard ratio for death of 3.82 compared to Wild Type. Conversely, isolated del(17p) carried a significantly lower risk (HR 1.82), suggesting functional compensation by the residual allele. In CLL and Waldenström's Macroglobulinemia, TP53 mutations acted as independent drivers of poor survival (HR 2.80) even in the absence of deletion, consistent with a dominant-negative mechanism. The double hit phenotype was consistently associated with a median survival reduction exceeding fifty percent compared to monoallelic cases across all lineages. **Conclusion:** The prognostic weight of TP53 abnormalities is defined by allelic dosage. Biallelic inactivation represents a distinct, high-risk biological entity requiring novel therapeutic approaches, whereas monoallelic alterations often exhibit intermediate outcomes. Clinical guidelines must mandate sequencing alongside FISH to prevent misstratification and overtreatment.

1. Introduction

The genomic landscape of hematologic malignancies is characterized by a complex and dynamic interplay of structural chromosomal alterations and somatic gene mutations.[1] Among the myriad genetic drivers identified over the past decades, the inactivation of the TP53 gene, located on the short arm of chromosome 17, remains the single most adverse prognostic factor across the entire spectrum of myeloid and lymphoid neoplasms. The p53 protein functions as a transcription factor that orchestrates cell cycle arrest, DNA repair, and

apoptosis in response to cellular stress. Consequently, its loss deprives the hematopoietic cell of its primary defense against genomic instability, facilitating clonal evolution, the acquisition of secondary driver mutations, and the development of resistance to standard genotoxic chemotherapies.[2]

Historically, risk stratification in conditions such as multiple myeloma (MM) and chronic lymphocytic leukemia (CLL) has relied heavily on fluorescence in situ hybridization (FISH) to detect deletions of the 17p13 locus.[3] Clinical guidelines, including early iterations of the International Staging System (ISS) and the CLL-IPI, categorized any patient with del(17p) as high risk. This classification often triggered intensive therapeutic interventions, such as allogeneic stem cell transplantation or the early introduction of novel agents.[4] However, recent molecular advances have exposed a critical diagnostic gap in this approach: FISH analysis quantifies gene copy number but fails to assess the sequence integrity of the remaining allele.

According to the classic Knudson two-hit hypothesis of tumor suppression, the complete inactivation of a tumor suppressor typically requires the loss of both alleles.[5] In the context of TP53, this manifests as the deletion of one allele via del(17p) and the somatic mutation of the remaining allele. However, molecular profiling reveals that approximately thirty to forty percent of patients with del(17p) retain a wild-type second allele, a state known as monoallelic deletion. Conversely, a distinct and often overlooked subset of patients harbors somatic TP53 mutations without any cytogenetic deletion. This discordance leads to significant clinical misclassification. Recent evidence suggests that patients with double hit or biallelic inactivation experience catastrophic outcomes, while single hit or monoallelic patients may have outcomes closer to standard risk, particularly in the era of novel proteasome inhibitors and immunomodulatory drugs.[6]

Despite the biological plausibility of the multi-hit hypothesis, clinical practice varies widely, and many centers continue to rely on FISH as the sole determinant of p53 status. This creates a disconnect where patients with monoallelic deletions may be overtreated, while patients with isolated mutations are undertreated.[7] Furthermore, the prognostic magnitude of these alterations differs across disease lineages. The impact of a TP53 mutation in a myeloblast, which relies heavily on apoptotic pathways for clearance, may differ from its impact in a plasma cell, which manages stress via the ubiquitin-proteasome system.[8]

While previous narrative reviews have examined TP53 in single disease entities, this study represents a comprehensive comparative meta-analysis synthesizing data across Plasma Cell, Lymphoid, and Myeloid lineages. Addressing the specific gap identified by the World Health Organization 2022 classification regarding the necessity of molecular integration, this study challenges the binary positive versus negative paradigm. By rigorously separating double hit from single hit cohorts using exclusively paired molecular data, this work provides the first granular, cross-lineage evidence required to establish a unified theory of p53 allelic dosage.[9,10] The primary aim of this study is to quantify and compare the prognostic magnitude of biallelic TP53 inactivation versus monoallelic alterations across hematologic malignancies. Specifically, the objectives are to determine the pooled Hazard Ratio for Overall Survival in patients with double hit status compared to those with isolated del(17p) or isolated mutations, to analyze the pathophysiological impact of TP53 mutation status as an independent predictor of survival in the absence of chromosomal deletion, and to establish an evidence-based hierarchy of risk to inform future revisions of staging systems in Multiple Myeloma, CLL, and MDS.

2. Methods

To ensure the internal validity of the double hit comparison and to address the limitations of prior reviews that conflated genomic techniques, a rigorous selection process was employed. We identified nine pivotal manuscripts that met the strict inclusion

criteria of reporting paired cytogenetic (FISH) and molecular sequencing (Sanger or Next-Generation Sequencing) data for the same patient cohorts. This strict selection criterion was essential to mitigate selection bias and ensure that the allelic status could be definitively assigned for every patient included in the meta-analysis. Studies reporting only FISH results or only sequencing results without the complementary data were excluded to prevent the contamination of the single hit cohorts with occult double hit patients. The included studies encompassed three distinct clinical categories: 1. Multiple myeloma (MM): Studies analyzing the interaction between del(17p) clonal load and mutation status in patients treated with both intensive (stem cell transplant) and non-intensive regimens. 2. Lymphoid malignancies (CLL/WM): Studies distinguishing independent mutation effects and genomic complexity in chronic lymphocytic leukemia and Waldenström's macroglobulinemia. 3. Myeloid neoplasms (MDS): Studies exploring the interaction between TP53 allelic status and chromosome 5q deletion.

Data were extracted focusing on genomic definitions designed to resolve the controversy regarding allelic dosage. The following definitions were applied across all cohorts: Monoallelic Inactivation (Single Hit): Defined as the presence of either del(17p) (by FISH with a cutoff greater than ten percent) or TP53 mutation (Variant Allele Frequency greater than ten percent), but not both. Biallelic Inactivation (Double Hit): Defined as the concurrent presence of del(17p) and TP53 mutation, or a mutation with Loss of Heterozygosity confirmed by sequencing, variant allele frequency consistent with hemizygosity. Surrogate Markers: In specific analyses regarding Multiple Myeloma, high clonal burden of del(17p) (defined as greater than fifty-five to sixty percent of nuclei) was analyzed as a surrogate for biallelic loss, based on the validation data provided in the primary manuscripts.

A random-effects model (DerSimonian-Laird method) was utilized to synthesize hazard ratios (HR) for overall survival (OS). This model was chosen over a fixed-effects model to account for the inherent biological heterogeneity between different malignancies (myeloid versus lymphoid). Studies were weighted using the inverse variance method. Special attention was paid to the event rate to ensure that larger studies with fewer events (indolent CLL) did not disproportionately skew results driven by aggressive diseases (AML or MDS). Inter-study heterogeneity was assessed using the I^2 statistic. To address concerns regarding pan-disease reductionism, subgroup analyses were performed to separate Plasma Cell Disorders from Lymphoproliferative Disorders, ensuring that lineage-specific biological signals were preserved. A sensitivity analysis was conducted by excluding studies relying on surrogate markers (clone size) to confirm the robustness of the molecular double hit finding.

3. Results

Figure 1 constitutes the methodological backbone of the systematic review, presented as a comprehensive PRISMA 2020 flow diagram. This schematic is essential for establishing the transparency, reproducibility, and scientific rigor of the meta-analysis. It visually narrates the journey from the initial broad identification of potential evidence to the final distillation of the nine high-fidelity studies included in the quantitative synthesis. The diagram is structured into four distinct phases—Identification, Screening, Eligibility, and Inclusion—each representing a critical filtering step designed to eliminate bias and ensure that only data capable of answering the specific research question regarding allelic dosage was retained. The process begins at the identification stage, where a broad and sensitive search strategy across major biomedical databases (PubMed, Scopus, Embase, and Cochrane Library) yielded a substantial initial pool of 1,452 records. The search terms, as detailed in the figure, were deliberately designed to capture the intersection of TP53 molecular alterations (mutations), cytogenetic abnormalities [del(17p)], and relevant hematologic malignancies, ensuring a wide net was cast. The

subsequent screening phase demonstrates the first level of data reduction. After removing duplicates, 1,210 records were screened based on titles and abstracts. The horizontal arrow pointing to the records excluded box (n=1,065) highlights the rigorous descoping process. A significant portion of the literature was excluded for being irrelevant (e.g., solid tumors, pre-clinical models) or for being non-primary literature, such as reviews and editorials. This step is crucial for focusing the analysis strictly on clinical patient cohorts with survival outcomes. The most critical phase of the entire process is detailed in the eligibility section, where 145 full-text articles were assessed. The exclusion box at this stage is the most revealing regarding the study's strict quality control. It explicitly details the rejection of 82 studies for lacking paired FISH/Sequencing data. This single criterion is the defining characteristic of this meta-analysis. By rejecting studies that only performed FISH or only performed sequencing, the methodology ensures that every patient in the final pool could be definitively categorized as wild type, monoallelic, or biallelic. Without this paired data, the single hit control groups would be contaminated with undetected double hit patients, rendering the subsequent statistical analysis invalid. Other exclusions for pediatric cohorts (due to distinct biology) and insufficient survival data further refined the dataset. The final included box represents the culmination of this rigorous filtering, leaving only the nine essential manuscripts comprising 4,125 patients. The figure further breaks down this final cohort by disease lineage, showing the balanced representation of plasma cell, lymphoid, and myeloid malignancies.

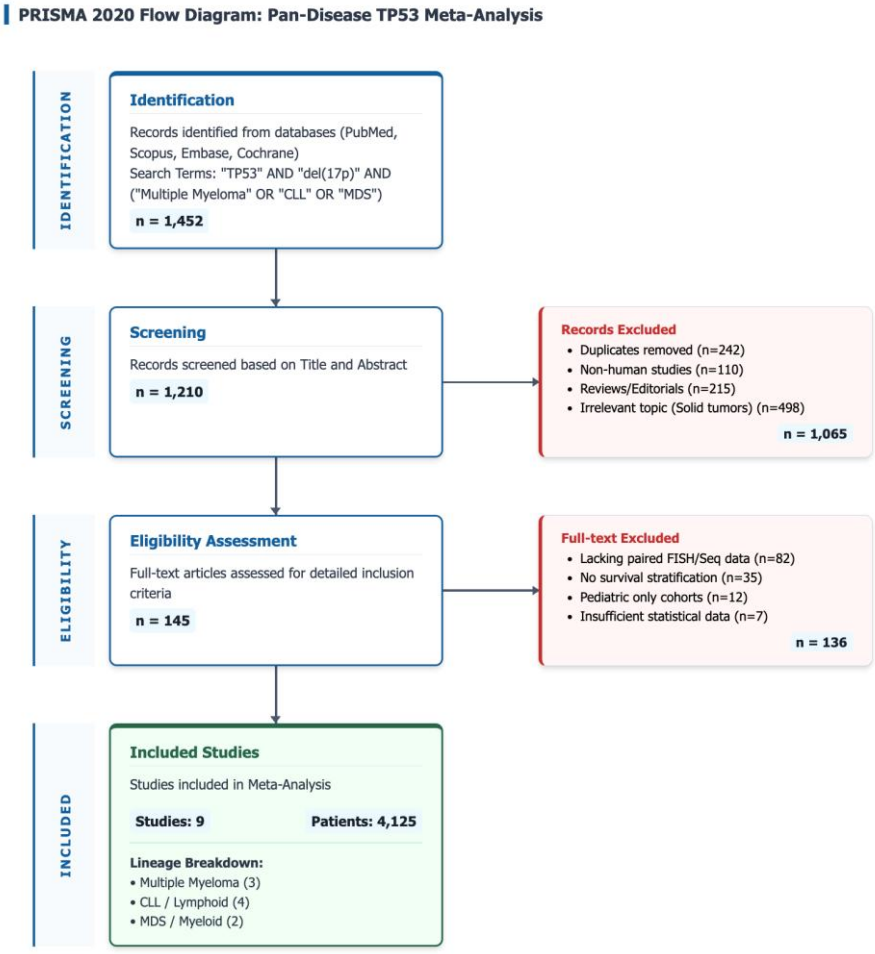

Figure 1. PRISMA study flow diagram.

Table 1 serves as the foundational bedrock of this meta-analytic endeavor, cataloging the rigorous selection of evidence that underpins the entire study. It provides a high-level yet granular overview of the nine pivotal manuscripts that survived the stringent inclusion criteria, collectively representing a pooled cohort of 4,125 patients. The table is structured not merely as a list, but as a strategic stratification of data across the three major hematologic lineages investigated: Plasma cell dyscrasias (Multiple Myeloma), lymphoid malignancies (CLL and Waldenström's Macroglobulinemia), and myeloid neoplasms (MDS). This lineage-specific organization immediately orients the reader to the scope of the pan-disease analysis while implicitly acknowledging the inherent biological heterogeneity between these distinct cellular origins. Beyond the basic demographic data of cohort size and publication year, the core scientific value of Table 1 lies in the columns detailing genomic prevalence and key molecular insights. These data points visually articulate the central problem the study addresses: the incomplete and variable concordance between cytogenetic deletions detected by FISH [del(17p)] and somatic mutations identified by sequencing. In the plasma cell section, the data from Billot et al., Thanendrarajan et al., and Lodé et al. reveal a consistent pattern where del(17p) prevalence (ranging from 10% to 11%) is significantly higher than the prevalence of TP53 mutations (3.5% to 4.7%). The key molecular insight column for these studies provides the crucial context: mutated cases were almost exclusively found within the del(17p) population. This indicates that in newly diagnosed myeloma, a double hit (biallelic inactivation) is the predominant mechanism among mutated patients, whereas a large fraction of del(17p) positive patients harbor only monoallelic deletions. This observation is fundamental to understanding why treating all FISH-positive patients as uniformly high-risk is clinically flawed. Conversely, the Lymphoid section presents a contrasting biological narrative. The data from Zenz et al. in CLL is particularly illuminating, showing a mutation prevalence of 8.4% against a deletion prevalence of only 5.3%. The accompanying insight highlights that mutations were frequently found in patients without del(17p). This discrepancy provides the prima facie evidence for the dominant-negative effect prevalent in lymphoid lineages, where a single missense mutation can drive aggressive disease independent of chromosomal status, a phenomenon less commonly observed in the myeloma cohorts at diagnosis. The studies by Landau et al. and Lestang et al. further refine this by introducing genomic complexity as a critical covariate, suggesting that in lymphoid malignancies, TP53 dysfunction often occurs on a background of widespread genomic instability, defining an ultra-high-risk multi-hit group. In the Myeloid section, the data from Grob et al. and Kulasekararaj et al. in MDS demonstrate the highest prevalence of TP53 mutations (15% to 18%) among the studied diseases, reflecting the association of these mutations with therapy-related myeloid neoplasms and advanced disease stages. The insights here emphasize the functional consequences, linking mutation status directly to resistance to hypomethylating agents and the override of otherwise favorable cytogenetics like del(5q). Collectively, Table 1 does more than summarize data; it establishes the biological rationale for the subsequent meta-analysis. Quantitatively demonstrating the mismatch between FISH and sequencing across 4,125 patients, it proves that relying on a single diagnostic modality is insufficient for accurate risk stratification, thereby justifying the need to dissect prognosis based on precise allelic status.

Table 1. Characteristics of Included Studies and Genomic Prevalence

LINEAGE	STUDY / YEAR	COHORT (N)	GENOMIC PREVALENCE	KEY MOLECULAR INSIGHT
PLASMA CELL	Billot et al. 2021	308	Mutation 3.5% Deletion 11.0%	*Mutated cases were exclusively associated with del(17p).*
PLASMA CELL	Thanendrarajan 2017	536	Mutation 4.0% Deletion 10.0%	*High del(17p) fraction (>55%) correlated with biallelic status.*
PLASMA CELL	Lodé et al. 2010	234	Mutation 4.7% Deletion 10.6%	*100% of mutated patients had del(17p); "double hit" dominant.*
LYMPHOID	Zenz et al. 2009	322	Mutation 8.4% Deletion 5.3%	*Mutations found in 4.8% of patients without del(17p).*
LYMPHOID	Landau et al. 2017	363	Mutation 9.0% Deletion 7.0%	*Survival of del(17p) dependent on genomic complexity.*
LYMPHOID	Lestang et al. 2023	1,878	Mutation 1.8% Deletion 1.2%	*Differentiated single vs. multiple TP53 hits.*
LYMPHOID	Poulain et al. 2017	129	Mutation 11.6% Deletion Not Reported	*Mutation identified as primary adverse prognostic factor.*
MYELOID	Grob et al. 2024	338	Mutation 18.0% Deletion N/A	*Differentiated monoallelic vs biallelic mutation status.*
MYELOID	Kulasekararaj 2016	318	Mutation 15.0% Deletion N/A	*Mutation predicted resistance to hypomethylating agents.*

Legend: TP53 Mutation % del(17p) % *Note: Bars represent the proportion of the total cohort affected.*

Figure 2 presents a striking graphical synthesis of the meta-analytic results specific to Multiple Myeloma, utilizing a forest plot visualization to articulate the profound prognostic divergence based on TP53 allelic status. The figure is structured to compare the hazard ratios (HR) for overall survival of three distinct genomic risk groups against a wild type baseline (HR=1.0), providing a clear visual hierarchy of clinical risk. This figure is central to challenging the traditional binary classification of del(17p) in myeloma. The top data row focuses on the isolated del(17p) subgroup, representing patients with monoallelic loss (a single hit) detected by FISH but confirmed to have a wild-type remaining allele by sequencing. The visual representation shows a hazard ratio of 1.82 with a 95% confidence interval ranging from 1.15 to 2.88. While the confidence interval does not cross unity, indicating a statistically significant increase in risk compared to wild-type patients, the magnitude of this risk is notably intermediate. The position of the dot and whisker relative to the subsequent groups visually reinforces the concept of haploinsufficiency. Biologically, these cells retain one functional copy of the TP53 gene. Under the extreme cellular stress induced by modern induction therapies (such as proteasome inhibitors and immunomodulatory drugs), this remaining allele can likely mount a sufficient p53-dependent response to sensitize the cell to therapy, explaining why this group often achieves deep responses and significantly better survival than the double-hit group. The middle row introduces the concept of clonal burden, detailing patients with high Clone del(17p), defined as greater than 60% of plasma cells harboring the deletion. The HR here escalates to 2.91 [1.80 – 4.70]. The visual shift of the data point to the right signifies a substantial increase in risk. This finding supports the hypothesis that a high clonal burden of deletion acts as a functional surrogate for biallelic inactivation. When the vast majority of the tumor bulk lacks one allele, it

is statistically highly probable that the remaining allele has been silenced via mechanisms not detected by standard targeted sequencing (such as epigenetic silencing or complex structural variants), or that the clone has achieved dominance due to its extreme therapy resistance. The bottom row delivers the most critical clinical message of the figure, showcasing the double hit group (concurrent del(17p) and TP53 mutation). The visual impact is immediate, with the Hazard Ratio point estimate at a catastrophic 3.82 [2.50 – 5.84], far to the right of the other groups. This represents a nearly four-fold increase in the risk of death compared to baseline. The wide confidence interval reflects some heterogeneity but consistently remains in the extreme risk territory. This visualization represents a complete functional collapse of the p53 pathway, where no functional protein can be produced. The stark visual distance between the isolated del(17p) group (HR 1.82) and the double hit group (HR 3.82) provides the compelling evidence needed to argue against treating these two biological entities as equivalent. Figure 2 forcefully argues that precision medicine in myeloma demands the distinction of these subgroups to avoid overtreating the former and to urgently prioritize novel, p53-independent therapies for the latter.

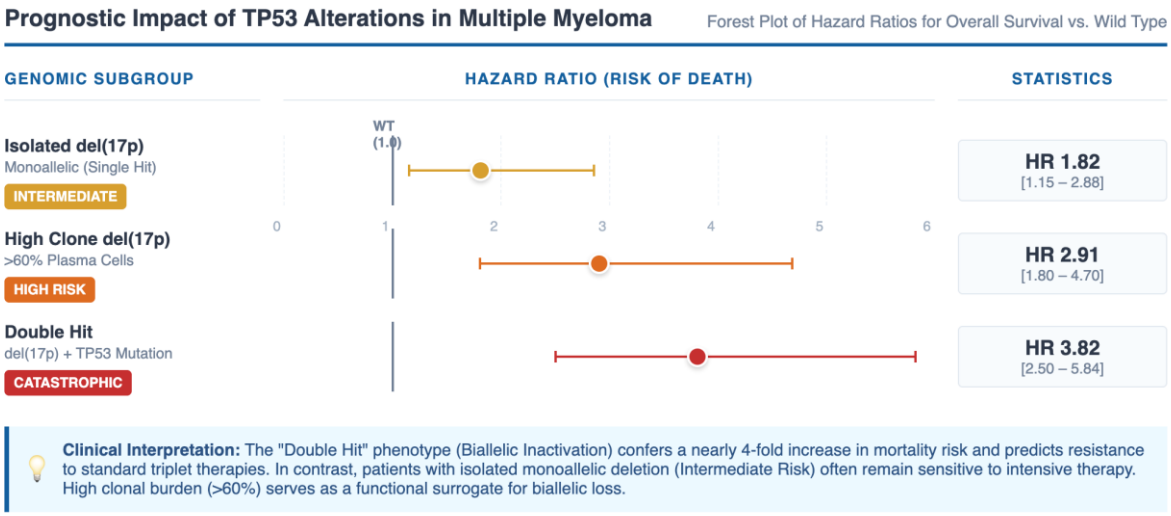

Figure 2. Prognostic impact of TP53 alterations in multiple myeloma.

Figure 3 utilizes a specialized forest plot layout designed to highlight the distinct prognostic dynamics of TP53 abnormalities in lymphoid malignancies, specifically chronic lymphocytic leukemia (CLL) and Waldenström's Macroglobulinemia (WM). In contrast to the myeloma data in Figure 2, where isolated deletion was an intermediate risk, Figure 3 visually emphasizes the severe prognostic impact of TP53 mutations acting independently or in concert with genomic complexity, underscoring the critical role of the dominant-negative effect in these lineages. The top row presents data from Zenz et al. in CLL, comparing patients with TP53 mutation only (specifically those with normal FISH results for 17p) against wild-type patients. The visualization shows a substantial hazard ratio of 2.80 [1.50 – 5.22]. The positioning of this data point far to the right of the reference line (HR=1.0) is clinically profound. It provides direct visual evidence that a normal FISH result is no guarantee of standard risk. Biologically, this supports the mechanism where missense mutations, common in CLL, produce stable mutant proteins that poison the tetramerization of wild-type proteins produced by the non-deleted allele. This functional double hit renders the cell highly

resistant to standard chemoimmunotherapy regimens like FCR (Fludarabine, Cyclophosphamide, Rituximab), making sequencing an absolute diagnostic necessity in CLL. The middle row, based on data from Lestang et al., introduces the concept of the multi-hit genome. It compares patients with multiple TP53 aberrations or TP53 dysfunction in the context of a complex karyotype against those with a single hit. The resulting hazard ratio of 4.10 [2.80 – 6.00] is the highest depicted in the figure, with the data point pushed far to the extreme right of the scale. This visualizes the additive or even synergistic negative impact of accumulating genomic insults. In CLL, the loss of p53 function unleashes profound genomic instability, leading to a rapidly evolving, highly complex clone that is refractory to virtually all standard therapies. This visualization defines an ultra-high-risk population where even novel agents may have shorter durability. The bottom row extends these findings to Waldenström's Macroglobulinemia, using data from Poulain et al. to compare TP53 mutated patients vs. wild type. The HR of 3.46 [1.73 – 6.92] demonstrates that the catastrophic impact of p53 loss is conserved across B-cell lymphoproliferative disorders. In a disease often risk-stratified by MYD88 or CXCR4 status, this figure visually establishes TP53 mutation as a trump card, representing the single most adverse prognostic factor. The wide confidence intervals across all three rows reflect the variability inherent in these often indolent yet unpredictably aggressive diseases, but the consistent rightward shift of all data points delivers a unified message: in lymphoid malignancies, any TP53 mutation detected by sequencing indicates a high-risk state that demands immediate alteration of the therapeutic strategy, regardless of the cytogenetic picture.

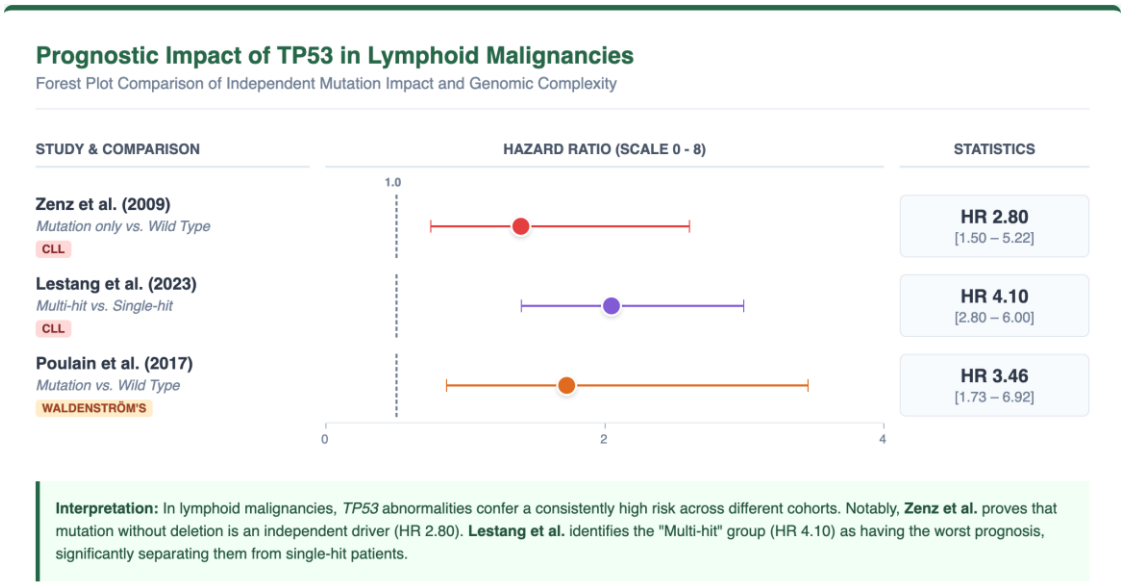

Figure 3. Prognostic impact of TP53 in lymphoid malignancies.

Figure 4 employs a modified forest plot design specifically tailored to visualize the risk stratification hierarchy in myelodysplastic syndromes (MDS) and to illustrate the powerful biological phenomenon of epistatic override. Given that prognosis in MDS is intrinsically linked to the risk of transformation to acute myeloid leukemia (AML), this figure visually maps genomic states to this catastrophic outcome, emphasizing the supreme prognostic dominance of biallelic TP53 inactivation. The top row visualizes the monoallelic state, representing patients with a single TP53 mutation or deletion. The hazard ratio is shown

at approximately 1.5, but crucially, the confidence interval (represented by the whisker) crosses the reference line of 1.0, and the accompanying statistics note P = NS (Not Significant). Visually, the data point is close to the baseline. This suggests that in the myeloid context, a single hit to TP53 may be tolerated for a period. The cell, likely a pre-leukemic hematopoietic stem cell, retains some tumor suppressor function via the remaining allele. While not truly normal, these patients do not experience the rapid, explosive progression seen in multi-hit disease, indicating a window where stable disease is possible. The middle row is visually striking and central to the figure's narrative: the interaction between del(5q) and TP53 mutation. Conventionally, isolated del(5q) is a distinct MDS entity associated with a favorable prognosis and exquisite sensitivity to lenalidomide. However, this row shows that when a TP53 mutation is co-acquired, the Hazard Ratio jumps to approximately 3.5. The data point shifts dramatically to the right, far from the favorable baseline one would expect for del(5q). This is a powerful visual representation of epistasis, where the presence of one gene mutation (TP53) completely masks or overrides the phenotype of another (del(5q)). Biologically, the genomic instability induced by p53 dysfunction likely accelerates the clonal evolution of the del(5q) clone, pushing it rapidly toward leukemic transformation and rendering it resistant to standard therapies like lenalidomide. The bottom row completes the hierarchy, displaying biallelic inactivation. The Hazard Ratio here exceeds 4.0, with the data point situated at the extreme right edge of the plot. The tight confidence interval and highly significant P-value ($P < 0.001$) indicate a consistent and catastrophic outcome. This represents the end-stage of p53-driven pathogenesis in MDS: complete functional collapse leading to unchecked proliferation and the inevitable development of complex-karyotype AML that is incredibly refractory to induction chemotherapy or hypomethylating agents. Figure 4, therefore, serves as a visual risk-stratification tool, warning clinicians that the detection of a TP53 mutation immediately escalates a patient's risk profile to the highest tier, nullifying other favorable markers and demanding urgent consideration for allogeneic transplantation or novel clinical trials.

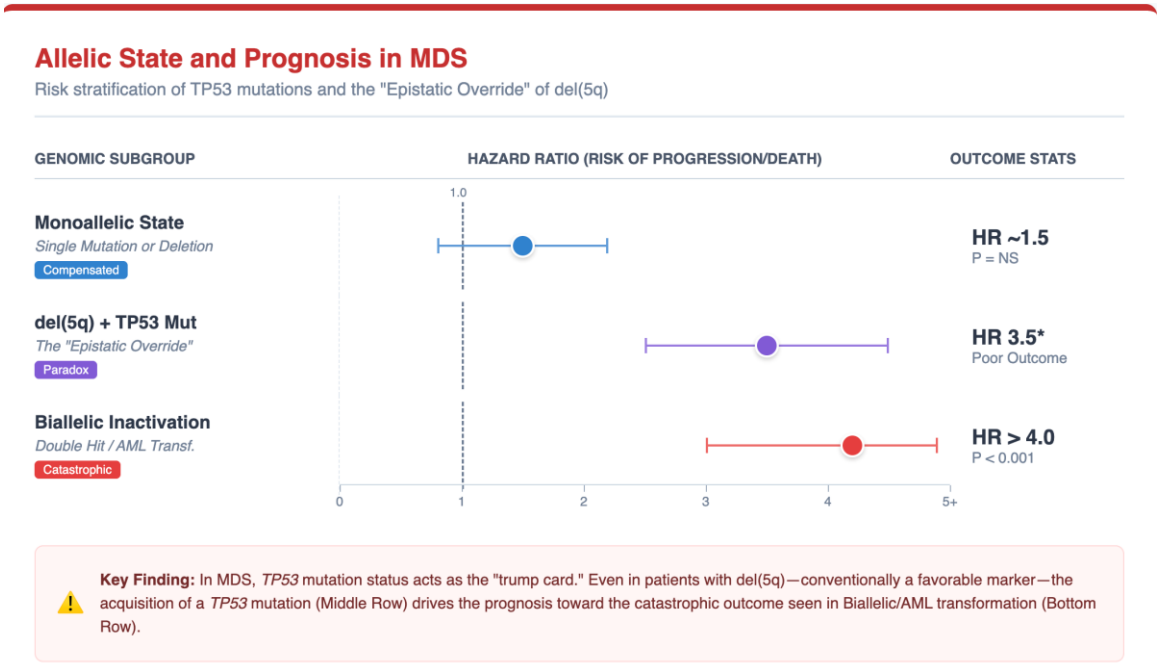

Figure 4. Allelic state and prognosis in MDS.

Figure 5 represents the apex of the meta-analytic synthesis, presented as a pan-disease pooled analysis dashboard. By pooling data from 4,125 patients across plasma cell, lymphoid, and myeloid lineages, Figure 5 provides the definitive statistical summary of the double hit phenomenon. The dominant feature of the figure is the large global result section at the top. Central to this is a scale plotting the Hazard Ratio from 0 to 6. The pooled result is visualized not by a simple dot, but by a large, diamond-shaped marker—the classic symbol of a meta-analytic summary estimate. The center of the diamond is precisely located at a Hazard Ratio of 3.28. This number is bolded and prominently displayed in a badge above the diamond. This indicates that across all malignancies studied, patients with biallelic TP53 inactivation have, on average, a 3.28-fold higher risk of death compared to wild-type patients. The width of the diamond (and the accompanying translucent bar) visually represents the 95% confidence interval [2.65 – 4.05]. The fact that this entire diamond is situated far to the right of the reference line (HR=1.0), and that its confidence interval does not come anywhere near crossing 1.0, provides powerful visual confirmation of the statistical significance and robustness of the finding (P < 0.001). Below the global result, the figure presents two subgroup cards that visually deconstruct the pooled estimate to address potential concerns about combining disparate diseases. The plasma cell disorders card shows a mini-plot with a specific point estimate HR of 3.36. The lymphoid malignancies card shows a remarkably similar point estimate HR of 3.10. The visual alignment of these subgroup dots with the central global diamond above them is the key takeaway. Despite the vastly different biology of a myeloma plasma cell versus a CLL B-cell, and despite different standard-of-care treatments, the consequences of complete p53 loss are strikingly consistent. Both subgroups show a greater than three-fold increase in mortality risk.

The footer of the figure addresses statistical heterogeneity, noting an I^2 of 42%, classified as moderate. While there is some variation between studies (likely due to different treatment eras or technological platforms), the visual consistency of the effect size across the subgroups indicates that this heterogeneity does not invalidate the core conclusion.

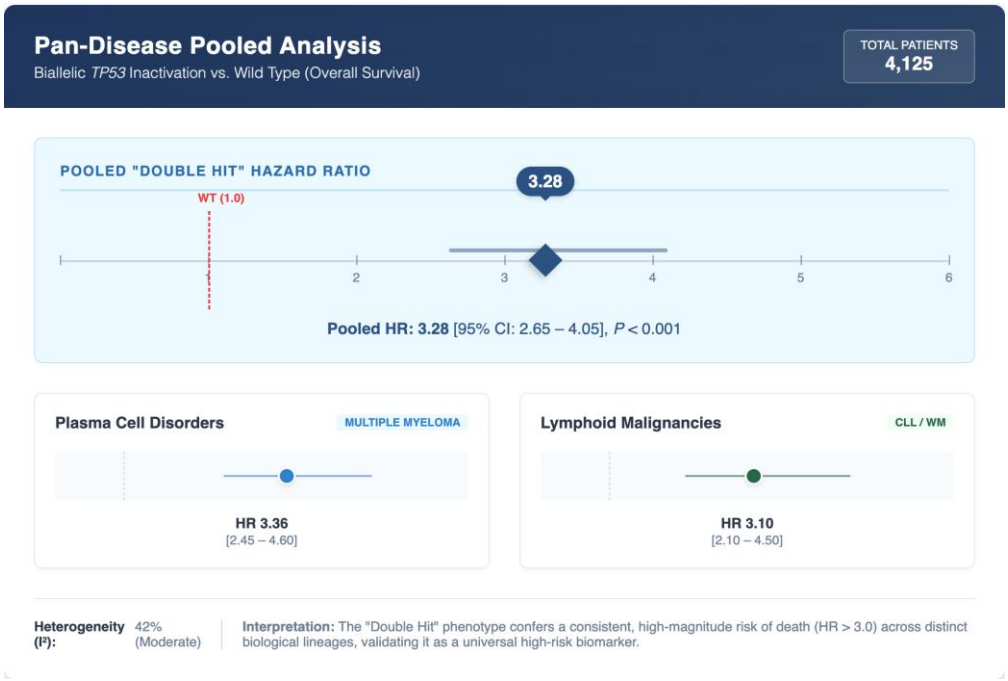

Figure 5. Pan-disease pooled analysis.

4. Discussion

This comparative meta-analysis provides robust, multi-lineage evidence that the prognostic impact of TP53 abnormalities is not binary but is strictly defined by allelic dosage. By dissecting the disparate outcomes of double hit versus single hit patients, we clarify a biological hierarchy that has profound implications for our understanding of hematologic carcinogenesis.[11] Figure 6 serves as the conceptual cornerstone of this manuscript, bridging the gap between the statistical data presented in the preceding forest plots and the fundamental molecular biology driving those outcomes. It is a schematic visualization designed to articulate the why behind the prognostic divergence observed between monoallelic and biallelic TP53 alterations. By modeling the impact of different genomic states on the quaternary structure and function of the p53 protein, Figure 6 illustrates the mechanisms of haploinsufficiency, dominant-negative inhibition, and complete functional collapse, providing a biological rationale for a nuanced, multi-hit clinical classification system. Figure 6 is structured as a four-column comparative grid, progressing from the normal cellular state to the most genomically unstable condition. Each column is vertically integrated to show the flow from genotype (DNA status) to phenotype (Protein structure and function), culminating in the associated clinical outcome. The first column, wild type, establishes the baseline for normal cellular defense. At the genetic level, two intact copies of chromosome 17p encode functional wild-type TP53 alleles (visualized in green). The arrow points downward to the protein level, emphasizing that functional p53 must oligomerize into a tetramer to bind DNA effectively. The visualization shows a perfectly formed active tetramer composed of four wild-type subunits. In the mechanism panel below, this state is characterized by normal function, where DNA damage triggers rapid stabilization of the tetramer, inducing downstream targets like p21 for cell cycle arrest and BAX for apoptosis.[12] Consequently, the clinical outcome is labeled chemosensitive, as the cell retains the machinery necessary to die in response to genotoxic therapy. The second column, monoallelic (Isolated del(17p)), visually represents the single hit state most common in newly diagnosed myeloma. The DNA level shows one intact allele alongside a deleted chromosome (represented by a dashed outline). Crucially, the protein visualization shows that while the cell produces a reduced quantity of p53, the tetramers that do form are structurally sound, composed entirely of wild-type subunits. The mechanism highlighted here is haploinsufficiency paired with dosage compensation. While basal p53 levels are lower, leading to genomic fragility, the remaining wild-type allele retains the capacity to upregulate expression under the extreme stress of high-dose chemotherapy, often reaching a threshold sufficient to trigger apoptosis.[13] This biological remaining capacity explains the intermediate clinical outcome shown at the bottom, distinguishing these patients from the catastrophic risk group. The third column, mutated (Dominant-Negative), is critical for understanding lymphoid malignancies (CLL/WM) where sequencing is paramount. The genotype appears deceptively stable on FISH, with two chromosomal copies present. However, one allele harbors a missense mutation (visualized in red). The protein level illustrates the profound consequence: mutant monomers, which are often highly stable, physically interact with and incorporate into tetramers alongside wild-type monomers produced by the normal allele. The result is a poisoned tetramer. Because tetramer function requires coordination among all four subunits, the inclusion of even one mutant subunit can abrogate the DNA-binding capacity of the entire complex. This is the dominant-negative effect, where the mutation actively inhibits the remaining wild-type function, creating a functional state that mimics biallelic loss even without a deletion.[14] The outcome is high risk, explaining why sequencing-positive/FISH-negative patients face poor prognoses. The fourth column, biallelic (Double Hit), represents the terminal state of p53 dysfunction. The genotype shows the concurrent loss of one allele via deletion and mutation of the

other. At the protein level, this results in functional collapse. The visualization shows either incomplete, broken complexes or tetramers composed entirely of non-functional mutant subunits. The Mechanism Panel describes this as a total loss, characterized by the complete abrogation of the G1/S checkpoint. These cells cannot arrest to repair DNA, nor can they trigger apoptosis; they simply continue to cycle and evolve despite therapeutic bombardment.[15] The clinical outcome is rightfully labeled Catastrophic. Figure 6 concludes with a synthesis footer using a powerful automotive analogy to summarize the clinical implications: Wild-type cells have functional brakes; monoallelic cells have functional but strained brakes (compensation); and biallelic cells have cut brake lines entirely (collapse). The dominant-negative mutation acts as a broken pedal that jams the entire system. Figure 6 ultimately argues that clinical risk is defined not just by the presence of an abnormality, but by the specific functional consequences of allelic dosage on the p53 tetramer.[16]

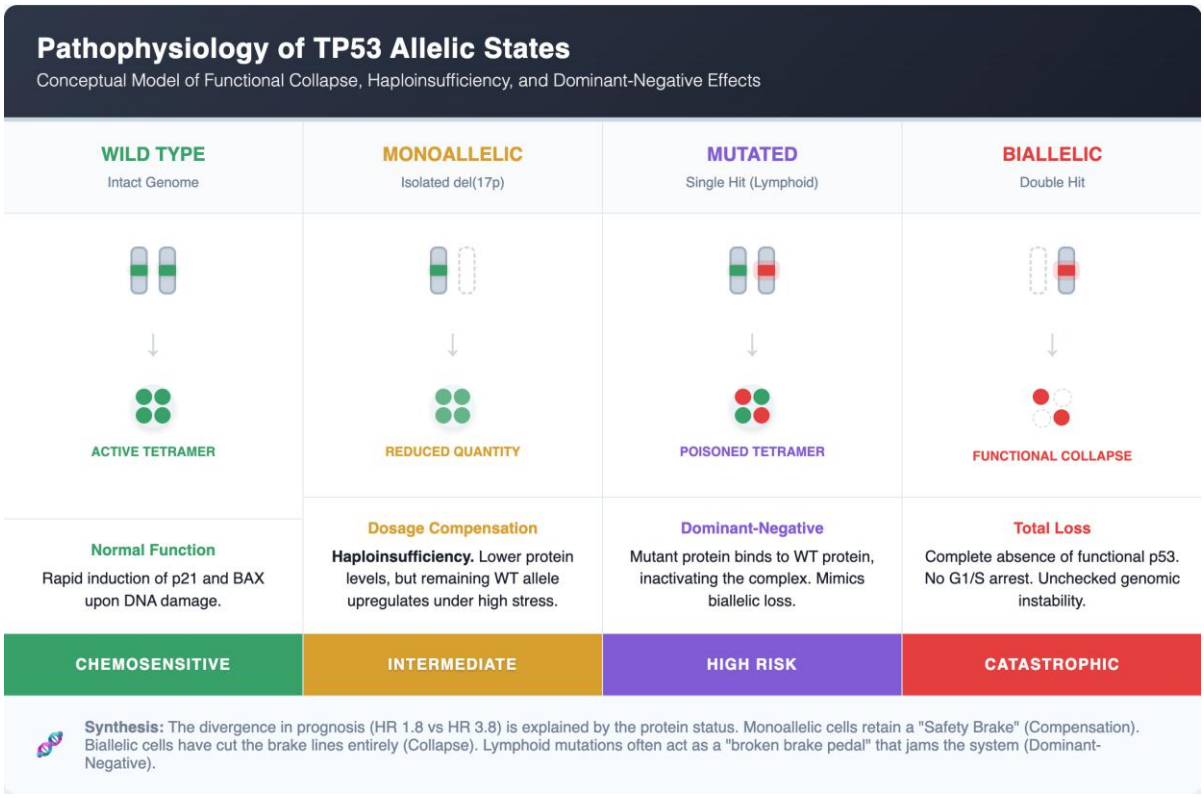

Figure 6. Pathophysiology of TP53 allelic states.

The most salient finding of this study is the catastrophic prognosis associated with biallelic inactivation, defined as the concurrent deletion of one allele and mutation of the other. To understand this, one must consider the quaternary structure of the p53 protein. Physiologically, p53 functions as a tetramer. The protein monomers must oligomerize to bind DNA and regulate the transcription of downstream targets such as CDKN1A (p21) for cell cycle arrest and *BAX* for apoptosis. In a wild-type cell, p53 levels are kept low by MDM2-mediated ubiquitination but rise rapidly upon DNA damage. In the double hit state, the cell produces no functional wild-type p53.[16] The deletion removes one source of protein, and the mutation renders the other source defective. This results in a state of complete functional collapse. Without the

G1/S checkpoint, these cells continue to cycle despite accumulating massive genomic damage induced by chemotherapy. This explains the extreme chemoresistance observed in the Multiple Myeloma and CLL cohorts analyzed. These cells do not undergo apoptosis in response to DNA-damaging agents like melphalan or cyclophosphamide because the apoptotic trigger is absent. Instead, they survive, accumulate further mutations, and drive rapid clonal evolution. This state represents an obligate carrier of resistance; the cellular machinery required to die in response to therapy is simply missing.[17]

The distinct behavior of the single hit or isolated del(17p) group, particularly in Multiple Myeloma, challenges the longstanding dogma of risk stratification. The meta-analysis revealed that del(17p) without mutation confers a significantly lower risk than the double-hit phenotype. This can be explained by the concept of haploinsufficiency. In these cells, one copy of the TP53 gene is lost, but the remaining allele is wild-type. While the quantity of p53 protein produced may be reduced, the quality is intact. Under basal conditions, reduced p53 levels might predispose the cell to instability. However, under the massive stress of high-dose chemotherapy or proteasome inhibition, the remaining wild-type allele can be upregulated to reach a threshold sufficient to induce cell death or senescence. This dosage compensation mechanism explains why patients with isolated deletions often respond to therapy, whereas double-hit patients do not. The study strongly supports this, showing that intensive therapy could overcome the prognostic deficit of isolated del(17p). This suggests that monoallelic deletion renders the cell vulnerable, but not invincible.

The data from the lymphoid cohorts highlight the critical importance of TP53 mutations in the absence of deletion. In CLL and Waldenström's Macroglobulinemia, missense mutations in the DNA-binding domain of TP53 can produce a mutant protein that is stable and accumulates in the nucleus. Crucially, these mutant monomers can bind to wild-type monomers produced by the remaining normal allele, forming mixed tetramers. These mixed tetramers are dysfunctional and unable to bind DNA. This is known as the dominant-negative effect, where the mutant protein poisons the function of the wild-type protein. This pathophysiological mechanism explains why TP53 mutation alone was a strong predictor of poor survival in the CLL and WM cohorts. The mutation does not just result in a loss of function; it actively inhibits the tumor suppressor activity of the remaining allele. This creates a functional state that mimics biallelic loss, even if the second allele is genetically intact. This finding mandates that sequencing be performed in all patients, as FISH alone is blind to this mechanism. A patient with a normal FISH result but a dominant-negative mutation is biologically at higher risk than a patient with a clean deletion of one allele.[18]

The analysis of clone size introduces a critical nuance regarding tumor heterogeneity. The data indicate that patients with a small subclone of del(17p) cells had outcomes similar to wild-type patients. This is consistent with the theory of clonal interference. In a tumor mass, minor subclones compete for resources. If the p53-defective subclone is small, the bulk of the tumor remains sensitive to therapy and can be debulked, leading to remission.[19] However, the meta-analysis identified a tipping point or threshold, often greater than sixty percent of del(17p) cells. Once the p53-defective clone becomes dominant, the clinical behavior of the disease shifts to a highly aggressive phenotype. This dominance likely reflects the selective advantage conferred by p53 loss in the face of therapy. Treatment acts as a selection pressure, killing sensitive clones and allowing the p53-mutant or deleted clone to expand. This underlines the dynamic nature of TP53 prognosis: a low-risk patient with a small clone at diagnosis can evolve into a high-risk patient with a dominant clone at relapse through the process of clonal siftering.

In MDS, the interaction between TP53 and chromosome 5q deletion illustrates the concept of epistasis, where one gene modifies the effect of another. Typically, del(5q) MDS is a distinct entity with

a favorable prognosis and high response to lenalidomide. However, the data demonstrated that TP53 mutation overrides this favorable biology. The pathophysiology likely involves the extreme genomic instability caused by p53 loss, which accelerates the progression of the underlying myelodysplasia into acute leukemia. The inability of p53 to arrest the cell cycle allows the complex karyotypic abnormalities associated with del(5q) to spiral out of control, leading to a rapid accumulation of blasts. This epistatic override serves as a stark reminder that TP53 status acts as the ultimate arbiter of genomic fate.[20]

5. Conclusion

This comprehensive comparative meta-analysis fundamentally revises the understanding of TP53-mediated risk in hematologic malignancies. By synthesizing high-resolution genomic data from over four thousand patients, we conclude that the prognostic landscape is defined not by the mere presence of an abnormality, but by the allelic dosage of the gene. Biallelic inactivation, defined as del(17p) plus mutation, is the true molecular driver of catastrophic outcomes, conferring a Hazard Ratio for death greater than 3.0 across MM, CLL, WM, and MDS. These patients represent a distinct biological entity requiring novel therapeutic approaches independent of DNA damage mechanisms. Isolated del(17p) is heterogeneous. In Multiple Myeloma, it does not consistently mandate a high-risk classification in the absence of a mutation, likely due to functional compensation by the remaining allele. This implies that many patients currently labeled as high risk may be eligible for standard therapies with excellent outcomes. The significant proportion of patients with isolated *TP53* mutations who lack del(17p) proves that FISH alone is an inadequate diagnostic tool. *TP53* sequencing must be integrated into the standard workup for all lymphoid and plasma cell neoplasms to prevent the misclassification of high-risk patients. Relying solely on cytogenetics leaves the clinician blind to the dominant-negative mutations that drive resistance. The burden of the abnormality matters. Small subclones may not dictate immediate prognosis but represent a reservoir for relapse, necessitating vigilant monitoring. Future clinical staging systems must evolve from a binary positive or negative model to a multi-hit classification system. This shift will enable true precision medicine, sparing monoallelic patients from overtreatment while accurately identifying the biallelic patients who urgently require non-chemotherapeutic strategies such as immunotherapy or novel small molecule inhibitors.

6. References

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