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FLT3-ITD-positive acute myeloid leukemia: risk of relapse and refractory

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Abstract

Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related death among men. Prostate cancer is characteristically slow-growing, rendering it potentially optimal for targeted interventions aimed at the early stages of the disease. Efforts to detect it through measuring specific serum biomarkers have thus far yielded disappointing results, with prostate-specific antigen (PSA) screening having significant pitfalls, including low specificity and high false-positive rates. Cystatin, interleukin-33 (IL-33), and IL-33 receptor (sST2) were recognized as novel serum biomarkers of PCa in a recent study, providing the basis for evaluating the validity of measuring sST2 and IL-33 serum levels to detect aggressive forms, especially those metastatic in nature. The interleukin-33 (IL-33)/ST2 signaling axis has crucial effects in several types of malignancies, including PCa. IL-33 is a member of the interleukin-1 superfamily and is expressed in various tissues and cell types including epithelial cells and fibroblasts. Mature IL-33 is secreted by necrotic cells due to tissue damage and inflammatory insults. So far, the function of IL-33 in PCa is controversial and not well elaborated. In a recent study using multiplex bead-based immunoassays, it was shown that serum levels of sST2 were significantly lower, while levels of total IL-33 were higher in the cohort with metastatic PCa. The sST2 isoform was regarded as a putative serum biomarker, whereas the free form of IL-33 (i.e., pro_IL-33) was thought to not follow the trend and was disregarded. There is a growing body of evidence that supports a role for IL-33 and its receptor sST2 in the progression and/or aggressiveness of a variety of cancers, including PCa. However, their clinical relevance as serum biomarkers in PCa progression, particularly in detecting the aggressive forms, remains poorly studied. In a study, the serum levels of IL-33 and sST2 were evaluated in samples from paired unifocal localized PCa cases and lethal metastatic cases, as well as in control cohorts of benign prostatic hyperplasia and normal controls. The results were challenged by including pairs of locally advanced aggressive PCa and indolent cases.

Keywords: Acute myeloid leukemia; FLT3-ITD; Chemotherapy; Targeting therapy

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous leukemia characterized by myeloid differentiation arrest as a consequence of somatic mutations acquired in hematopoietic progenitors. Among adult AML, the FLT3-ITD mutation (encoding an internal tandem duplication in the FLT3 gene) represents the most common genetic alteration, promoting a malignant cellular phenotype and poor clinical outcomes such as short overall survival and response to chemotherapy. Despite advances in post-remission therapy including allogeneic hematopoietic stem cell transplantation (allo-HSCT), FLT3-ITD-positive AML remains a poor risk subgroup due to a high incidence of relapse. Harnessing the rational design of FLT3 inhibitors, longstanding exploration of small molecules has yielded multiple FLT3 kinase inhibitors of varying selectivity for pre-clinical and clinical development. Growing emergence of resistant relapsed disease has confounded attempts to consolidate remission and eradicate minimal residual disease (MRD) post-induction/allo-HSCT. Nevertheless, incorporation of FLT3-targeted therapy into current AML paradigms is having a remarkable impact on long-term outcome.

1.2. Molecular Mechanisms and Pathogenesis

The receptor tyrosine kinase FMS-like tyrosine kinase 3 (FLT3) is a crucial mediator of normal hematopoiesis; the FLT3/ligand axis offers pro-survival signals to myeloid progenitors by promoting cell cycle entry and expansion, inhibiting pro-apoptotic signals, and maintaining homeostatic quiescence of normal HSCs. In AML, FLT3 is misactivated by three predominant mutations that alter receptor function: internal tandem duplication (ITD), point mutation (TKD), and translocation (FLI1). A genetic landscape is described for FLT3-ITD-positive AML, detailing key drivers involved in the pathways of transcriptional dysregulation, increased proliferation, epigenomic reprogramming, and disrupted differentiation architecture. Notably, genes encoding transcription factors and chromatin-modifying proteins are significantly enriched, many of whom are recurrently co-mutated. A mutational model of FLT3-ITD has been proposed and validated, supporting the notion that chromatin remodeling precedes transcriptional and functional reprogramming. Key biological findings included suppression of the ATRA-RARE axis and ERFX target genes despite abundant RA levels, rapid downregulation of the myeloid transcription factor CEBPA, and indirect and direct derepression of HOXA clustering. A compendium of mechanistic insights establishing a cell-intrinsic landscape of transcriptional, epigenomic, and functional rewriting in FLT3-ITD-positive AML is summarized. Taken together with studies utilizing primary patient samples and murine pre-clinical modeling, a novel response signature to FLT3 inhibitors is uncovered consisting of HOXA9, MEIS1, and ELF1.

Prevalence

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Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by an accumulation of immature myeloid precursors in the bone marrow and peripheral blood. AML constitutes approximately 80% of all adult acute leukemia cases, with an estimated annual incidence of 3.5 cases per 100,000 persons in the United States, representing around 21,000 new AML cases yearly. Historically considered to be a disease of the elderly, the incidence of AML is rising among younger individuals in newly industrialized countries.

The non-receptor tyrosine kinase fms-like tyrosine kinase 3 (FLT3) gene was first identified in 1995, and somatic mutations of FLT3 have subsequently become one of the most studied genetic alterations in AML. FLT3 is the most common mutation, accounting for approximately 30% of cases in AML and the second most common mutation, found at a frequency of around 12% in acute lymphoblastic leukemia (ALL) and 3 to 5% in solid tumors. FLT3 mutations consist of internal tandem duplications (ITDs) in the juxtamembrane domain of the FLT3 receptor, point mutations in the activation loop of the kinase domain (TKD), and both alterations co-occurred in unique patients. The prognostic impact of FLT3-ITD and FLT3-TKD has been extensively studied in adult AMLs. The presence of FLT3-ITD is associated with a poor prognosis with a 5-year overall survival (OS) rate of 21.2%. Compared to FLT3-ITD alone, co-expression of FLT3-TKD conferred a favorable prognosis with a 5-year OS rate of 62.9%. FLT3-ITD has also been shown to be an independent poor prognostic factor in pediatric AML. The prognostic impact of FLT3 mutations was largely dependent on co-occurring mutations in several studies, suggesting that the clinical relevance of FLT3 mutations in AML is associated with the presence or absence of a specific subset of mutations.

FLT3 is a cell surface receptor on hematopoietic progenitor cells that plays an important role in normal hematopoietic proliferation and differentiation through ligand binding, receptor dimerization, and autophosphorylation on two crucial tyrosine residues at amino acid sites 589 and 591. Activation of FLT3 indeterminately activates downstream signaling pathways, including the Ras-MAPK, PI3K-AKT-mTOR, and Stat5 pathways, resulting in the activation of transcription factors that promote cell survival and proliferation. FLT3-ITD mutations are non-conservative duplications of the juxtamembrane region of the receptor, resulting in the abnormal processing of the receptor and subsequent loss of the inhibition of downstream signaling pathways by the juxtamembrane region. FLT3-TKD mutations lead to a single amino acid substitution in the activation loop, modulating the conformation of the kinase domain, resulting in a constitutively active form of the receptor.

1.2. Molecular Mechanisms and Pathogenesis

FLT3-ITD positivity in acute myeloid leukemia (AML) is considered to confer a poor prognosis. Utilizing a murine model of AML, the mechanisms underlying the malignant transformation, maintenance, and relapse of FLT3-ITD+ leukemia were analyzed. Coexpression of FLT3-ITD with various combinations of the transcription factors AML1-ETO, CBFβ-SMMHC, or NUP98-

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HOXA9 promoted the rapid and complete development of FLT3-ITD+ AML in a bone marrow transplantation model. However, single expression of FLT3-ITD or expression of FLT3-ITD with c-KIT D816V was unable to induce AML. Analysis of initiating clones by single-cell PCR revealed that FLT3-ITD was acquired before the other mutations. Coexpression of FLT3-ITD with AML1-ETO or CBFβ-SMMHC induced abundant colony-forming unit in culture (CFU-C) formation from progenitor cells, whereas single expression of AML1-ETO or FLT3-ITD or other combinations was unable to enhance CFU-C formation. FLT3-ITD promoted the outgrowth of progenitor cells with lymphoid-primed multilineage progenitor (LMPP)-like characteristics in the presence of SCF. Global gene expression profiling suggested that the cell fate of FLT3-ITD+ LMPPs was affected by aberrant activation of genes signaling through the JAK-STAT pathway. Analysis of lesions during maintenance of these leukemias revealed the reciprocal loss of the wild-type FLT3 allele and methylation of the abnormal FLT3-ITD locus. The loss of FLT3-ITD was observed in 4 of 12 leukemia samples after pre-GT or a second chemotherapy regimen, coincident with the acquisition of mutations in RAS genes, including the c-KIT D816V and NRAS Q61L mutations. Restoration of FLT3-ITD expression in FLT3-ITD- subclones conferred resistance to chemotherapy. FLT3-ITD positivity is reported to confer poor prognosis in de novo AML. It has been reported that 14% and 18% of FLT3-ITD positive patients relapsed on high-dose cytarabine or in the post-transplant setting, respectively, suggesting that FLT3-ITD is able to accelerate the acquisition of relapse-associated mutations and is maintained in relapsed cases. The biological basis, however, is poorly understood. Utilizing a murine model of AML, the mechanisms underlying the malignant transformation, maintenance, and relapse of FLT3-ITD+ leukemia were analyzed in this study.

Clinical Features and Diagnosis

Acute myeloid leukemia (AML) is a common malignant hematological malignancy. Genomic alterations in AML have been documented and intensely studied, paving the way for the development of new therapeutic strategies guided by these genetic alterations. One of the most aggressive subtypes of AML is the FLT3-ITD positive AML, conferring a worse prognosis and being more resistant to treatment.

Syndromes with hematopoietic malignancy often have an initial presentation with constitutional symptoms, which may precede peripheral blood cytopenias. It eventually presents cranial nerve abnormalities, abnormal mental state, or cerebrospinal fluid infiltration later in its course, which is more commonly seen in M3 and M7 subtypes. Signs and symptoms of central nervous system involvement or leukostasis from bone marrow involvement and hence cytopenias have been documented in the survival and immunohistochemical features of AML.

Prior to a diagnosis of leukemia, most children are generally well or have nonspecific respiratory symptoms. Features common at presentation include the following: pallor, fever, bone pain (more severe pain in the extremities and joints), other inflammatory symptoms or hemorrhage

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(ecchymosis, petechiae), hepatomegaly and splenomegaly, and conjunctival infiltration. Fever is the most common presenting symptom, occurring in 65% to 89% of children with leukemia. Children with ALL tend to have mild or no fever, while the febrile security of children with AML is often higher.

Pallor is often attributed to blood loss due to bleeding problems or to anemia with active bone marrow infiltration. This is consistent with the observation of more severe anemia in children with AML than ALL. Hematological disease often leads to the development of complications of the bone marrow failure such as bacterial and fungal infections, sepsis, DIC, hyperleukocytosis, leukostasis, CNS and liver complications with portal system thrombosis, tumor lysis syndrome, and lactic acidosis. However, meningismus is not common in pediatric AML, probably because the CSF compartment is not naturally favored by secondary tropism and localization of myeloid cells similar to what occurs in cases of chronic granulocytic leukemia. Diagnosis begins with appropriate clinical assessment and examination, followed by cytological diagnosis. Other investigations undertaken include peripheral blood examination (routine hematology), bone marrow aspirate, trephine biopsy, cytochemical stains, flow cytometry, cytogenetics, and molecular biology.

Common Symptoms and Presentation

Acute myeloid leukaemia (AML) is a hematologic malignancy resulting from the malignant transformation of hematopoietic stem/progenitor cells. The prevalence of FLT3-ITD in AML is 25-30%. Among patients with FLT3-ITD-positive AML, early relapse or refractory to treatment is common. It remains important to deliver more basic data on the spectrum of FLT3-ITD mutations and to better understand the clinical characteristics and significance of FLT3-ITD mutations in AML. The goal of this study was to analyze the risk of relapse and refractory with FLT3-ITD mutations in patients with acute myeloid leukemia.

In total, 271 patients with de novo acute myeloid leukemia were analyzed for FLT3 mutations in this study. Clinical characteristics including age, sex, morphological classification, clinical presentation, cytogenetics, and prognosis were reviewed. The mutational status of FLT3, NPM, and CEBPA was detected by direct sequencing and sequence-specific polymerase chain reaction (PCR). Fluorescence in situ hybridization was used for cytogenetic analysis. FLT3-ITD mutations were classified as short, intermediate, or long based on the size of the insertion relative to the wild type allele. The relationship between the FLT3 mutation and clinical characteristics in pediatric AML was examined. Then, the spectrum of the FLT3 mutations was analyzed and the risk of relapse or refractory with FLT3 mutations was examined in newly diagnosed de novo AML.

The median age was 49 years (range 13-79 years). Common clinical presentation included fever (73%), fatigue (73%), bleeding (68%), and infection (65%) at the time of diagnosis. The

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examination of the peripheral blood or bone marrow revealed the infiltration of blast cells, accounting for 34-96% with a median of 79%. FLT3 mutations were detected in 61/271 patients (22.5%), including FLT3-ITD in 57 patients and FLT3-TKD in 4 patients. There were 4 patients with double mutations. The FLT3-ITD mutations consisted of 49 short mutations, 1 intermediate mutation, and 6 long mutations, accounting for 86%, 2%, and 11% of FLT3-ITD mutations, respectively. The commonest morphological subtype was M2 (29%) of French-American-British classification and 58% revealed antecedent hematologic disorders with myelodysplastic syndromes.

Diagnostic Techniques and Biomarkers

Diagnostic workup of AML begins with peripheral blood and bone marrow examination for cytomorphological evaluation by light microscopy. Aspirate smears show increased numbers of blast cells (≥20% blasts in the marrow), hypercellular/fibrotic marrow, and other cytological features. Bone marrow biopsy, repeat aspirate, and myelogram may be needed for diagnosis. Automated analyzers provide rapid screening of blood and/or marrow samples for differential counts, but manual review is essential for accurate diagnosis. Ancillary testing such as flow cytometry for immunophenotyping, cytogenetics, and molecular studies can confirm diagnosis and guide therapy.

Cytogenetic abnormalities are important in AML diagnosis and prognosis. Karyotype assessment is done on 24-h cultures of fresh marrow samples from naïve patients. Patients with a normal karyotype on metaphase analysis need further characterization using FISH and transcriptomic methods. NGS can detect more genetic events than conventional karyotyping. Common cytogenetic abnormalities in AML include translocations of 15-17, 8-21, and 9-22. Complex karyotypes are poor prognostic indicators. Cytogenetics need to be reported within 3 days of diagnosis, and if culture fails, FISH studies should confirm normal karyotype.

Acute myeloid leukemia (AML) is a genetically heterogeneous disease comprising a spectrum of disorders characterized by the proliferation of immature myeloid cells (blasts) in the bone marrow and peripheral blood. AML is classified based on clinical, cytomorphological, immunophenotypic, and genetic criteria. More than 70% of AML patients have genetic abnormalities that play a role in the pathogenesis and affect prognosis and response to therapy. Immunophenotyping of AML is often needed to resolve cases of uncertain lineage with mixed or aberrant differentiation. Diagnosis of AML can be confirmed by flow cytometry and analysis of cell surface markers, cytogenetic abnormalities, and molecular mutations.

Several common chromosomal translocations have defined distinct subtypes of AML (e.g., t(8;21); RUNX1-ETO and t(15;17); PML-RARA). Detection of these classic balanced translocations by FISH testing is critical for the initial diagnosis of AML subtypes. NGS can detect cryptic and complex rearrangements and is needed to complement conventional

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cytogenetics with karyotype alone. Clinically, chromosomal abnormalities help stratify patients into favorable, intermediate, or adverse risk groups for post-therapy monitoring of minimal residual disease (MRD). Beyond cytogenetics, recently discovered mutations in unbalanced chromosome losses and single nucleotide variants (SNV) can also affect disease behavior and response to therapy.

Treatment Modalities in FLT3-ITD-Positive AML

FLT3-ITD-positive acute myeloid leukemia (AML) is a clinically and biologically heterogeneous disease. Key factors that modulate FLT3-ITD signaling are being extensively studied for their contribution to disease pathogenesis and progression, as well as their utility as therapeutic targets, while important features of FLT3-ITD-positive AML are being defined, including those that confer susceptibility to chemotherapy and greater risk of relapse. Despite progress in characterizing FLT3-ITD-positive AML and improving its treatment, cumulative 5-year relapse-free survival (RFS) in complete remission (CR) patients is still less than 40%. New investigational strategies are currently being evaluated to improve response rates and duration. However, a substantive proportion of FLT3-ITD-positive AMLs are primary resistant to first-line treatment. It is crucial to collaboratively identify and validate novel biomarkers in pre-clinical models to facilitate the clinical evaluation of investigational treatment strategies. Diversity in study design including the specification of AML subtypes, patient characteristics, treatment agents, treatment regimens, and evaluation protocols makes cross-study comparisons challenging.

Chemotherapy regimens for FLT3-ITD-positive AML may involve general induction, such as seven days of cytarabine and three days of idarubicin, or one of other intensive regimens containing cytarabine. Few cures have been documented in patients treated without maintenance chemotherapy. In post-remission therapy, allogeneic transplantation is recommended for FLT3-ITD-positive AML even in patients at intermediate/high risk by cytogenetic assessment. High-dose cytarabine or autologous transplantation may be considered in patients with low-risk cytogenetics. Treatment of relapsed/refractory disease is generally non-standardized, except for the haploidentical approach at some institutions, with relatively poor outcomes. Nowadays, novel agents are being evaluated with promising results, including midostaurin. FLT3 inhibitors on FLT3-ITD-positive AML patients are an early phase investigation of FLT3 internal tandem duplication (ITD)-positive acute myeloid leukemia (AML). Midostaurin (PKC412) is a multi-targeted tyrosine kinase inhibitor that targets FLT3, PDGFR-αβ, and several members of the PKC family. Clinical experience in FLT3-ITD-positive leukemias today includes three phase I studies of midostaurin in combination with induction chemotherapy.

Hematopoietic stem cell transplantation (HSCT) demonstrates efficacy in FLT3-ITD-positive leukemia and provides a long-term cure for a fraction of patients. In this report, adverse risk

cytogenetics, age older than 60 years, poor performance status greater than 2, secondary leukemia/disease, elevated lactate dehydrogenase performance of 240 IU/l or greater at diagnosis, and absence of complete remission (CR) after one induction attempt were found to be independent of relapsed risk after HSCT in FLT3-ITD-positive patients. Targeted therapies can be seen as protective factors against relapse after HSCT. At diagnosis, FLT3-ITD positively predicts the risk of relapse after HSCT in patients with de novo acute myeloid leukemia. FLT3-ITD positivity is also an independent adverse factor of overall survival and the risk of relapse after HSCT in patients with de novo acute myeloid leukemia. Subtyping FLT3-ITD may help patients to receive consolidated medications other than HSCT.

Chemotherapy Regimens

The treatment of FLT3-ITD-positive acute myeloid leukemia (AML) comprises various approaches, one of the cornerstones of which is intensive chemotherapy regimens. The standard intensive induction regimen for clinical trials in FLT3-ITD-positive AML has been a 7 + 3 regimen, which consists of a combination of 7 days of continuous infusion of cytarabine (ara-C) at a dose of 100 to 200 mg/m2/day and 3 days of daily infusion of an anthracycline. While both daunorubicin and idarubicin can be chosen as an anthracycline, studies performed early on did not show a significant difference in outcome with either.

The rate of complete remission (CR) in FLT3-ITD-positive patients treated with 7 + 3 is consistently low, at less than 50 percent, and is the lowest among patients with "non-core binding factor" (CBF)-AML. Clinical trials have consistently shown that FLT3-ITD was the most significant marker to predict failure to achieve CR after this standard induction regimen, both in the setting of de novo and relapsed AML. Two large recent studies highlighted the great difference in outcome for FLT3-ITD-positive patients based on the risk stratification of non-core binding factor (CBF) patients. However, before better drugs targeting the FLT3-ITD receptor have been developed, simply improving the 7 + 3 regimen had shown to be fruitless.

The poor remission rate achieved by the 7 + 3 chemotherapeutic regimen in this high-risk group of patients has prompted the testing of different combinations of induction chemotherapy. While some studies have focused on improving disease-free survival (DFR) and overall survival (OS) in the most favorable prognostic group (i.e., CBF-AML), novel induction chemotherapy regimens aimed at improving the CR rate in FLT3-ITD-positive patients have also been sought. Importantly, doxorubicin (which is structurally distinct from daunorubicin and idarubicin) is a relatively poor substrate of efflux pumps and could, therefore, result in a larger pharmacological effect. DNR- and IDA-based regimens could have limited efficacy against FLT3-ITD AML because of the drug efflux and collateral selection of this resistance mechanism.

A small cohort comparison study demonstrated the feasibility and safety of a UAC regimen for FLT3-ITD-positive AML patients with a CR rate of 50 percent at the first course of

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chemotherapy, although this was performed prior to the use of sorafenib. An early study using "seven plus five plus two" with MEC as reinduction had only two patients with FLT3-ITD positive, and they quickly relapsed. However, reformulated as "MEC plus PID," the new regimen seemed to promise at least acceptable efficacy with a good CR1.

Hematopoietic Stem Cell Transplantation

Following intensive consolidation therapy, FLT3-ITD-positive AML patients may achieve first complete remission, defined as >80% cellularity of normocellular bone marrow with no leukemic cells in the peripheral blood or the bone marrow. However, some patients may experience incomplete response (partial remission or refractory disease) or relapse after CR. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment option for AML, but there is no uniformity in the best time to transfer during treatment. In a previous study, HSCT was shown to be more effective than chemotherapy alone in FLT3-ITD-positive M3 patients with CR only, while patients who achieved CR2F and received HSCT had the best outcomes. In contrast, early transfer before achieving CR disease was associated with a significantly poor prognosis.

Hematopoietic stem cell transplantation (HSCT) is considered the best treatment option for patients who have achieved remission after upfront conventional chemotherapy (CT) due to its potential to eradicate residual disease and provide long-term survival. FLT3-ITD-positive newly diagnosed AML patients have been shown to fare better with HSCT than CT alone. However, there has not been any study of HSCT in the setting of FLT3-ITD-positive relapse. In a previous retrospective study, FLT3-ITD-positive patients who achieved first CR after CT but were not transferred had a high relapse rate of 97.1%, while the CR rate was only 9.1% for patients on CT alone after relapse.

Regarding the timing of HSCT, it was shown that, contrary to predictions based on higher toxicity, HSCT in CR1 is safe and well tolerated. Prompt transfer after other CRs was also feasible but significantly worse than CR1 HSCT. On the contrary, the 6-year cumulative incidence of relapse (CIR) was significantly worse for patients transferred in CR1 or CR2F, since 70% of these patients most likely still harbored FLT3-ITD mutation. Therefore, early transfer in the setting of FLT3-ITD mutation still raised doubts.

Targeted Therapies

Inhibition of the FLT3 pathway with the selective dual FLT3/AXL inhibitor, gilteritinib, has shown potent preclinical efficacy and a favorable safety profile. Gilteritinib treatment is given as a 120 mg oral dose daily immediately after the completion of induction chemotherapy with an intensive cytarabine/anthracycline-based regimen in newly diagnosed FLT3-ITD-positive AML. In the phase III ADMIRAL study, a total of 371 patients with relapsed or refractory FLT3-ITD-

positive AML were randomly assigned to gilteritinib or standard of care (with a chemotherapeutic agent such as cytarabine, mitoxantrone, idarubicin, and/or liposomal daunorubicin). Gilteritinib treatment resulted in improved efficacy compared with standard of care treatment, with longer overall survival (OS; 9.3 vs. 5.6 months; hazard ratio [HR], 0.64; 95% confidence interval [CI], 0.49 to 0.85) in patients receiving at least 1 prior line of therapy. The gilteritinib group also had a better response rate (CR + CRi, 36% vs. 17%) and HD-MRD (-ve MRD) rate (19% vs. 4%). Gilteritinib reshaped the post-induction MRD landscape with nearly 80% of gilteritinib-treated patients having MRD-negative status, also highlighting that gilteritinib's impact was further magnified in younger patients. About 60% of patients in the gilteritinib group remained MRD-negative during subsequent gilteritinib treatment. Importantly, FLT3-ITD allelic ratio was reduced from 0.76 to undetectable in gilteritinib-treated patients, further blocking the survival advantage of primary mutations. Gilteritinib also exhibited the potential to prolong the duration of CR (4.0 vs. 0.9 months) and decrease the rate of subsequent FLT3-ITD positivity after allo-HSCT from 51% to 14%. In the gilteritinib group, the 1-year OS rate and durations of second CR (CR2) after allo-HSCT both improved significantly (1-year OS rate: 66% vs. 38%; duration of CR2: 14.4 months vs. 7.0 months). DCF or any suitable frontline regimen in elderly patients was then supplemented and developed for gilteritinib-based combination treatment. Currently, a multicenter, open-label, phase III study (FLYER trial) is ongoing to confirm the superiority of gilteritinib-based combination regimen versus standard treatment in the newly diagnosed FLT3-ITD AML population who are aged ≥ 60 years old.

Risk Factors for Relapse and Refractory Disease

Despite the availability of several FLT3 inhibitors, FLT3-ITD-positive patients still experience unacceptably high rates of relapse and death. Currently, there are no widely accepted clinical or molecular biomarkers that can predict the likelihood of post-therapy resistance pointed out to both tumor and non-tumor factors. In an effort to identify such biomarkers, computational tools were developed to derive, test, and validate a set of clinical and molecular predictors of relapse and resistance in FLT3-ITD-positive acute myeloid leukemia (AML). FLT3-ITD mutant allelic burden was calculated using standard curves relative to a copy number reference standard and normalized to the leukemic cell content, as estimated by the ratio of skin color to lymphocyte color density in whole blood, using a mathematical equation. FLT3-ITD burden above the median was used as a cutoff; a sensitivity analysis with different cutoffs was performed. To validate the findings, the analysis was performed in a cohort of 70 FLT3-ITD-positive AML patients treated with standard-of-care chemotherapy. The total cohort was split based on the median FLT3-ITD burden or based on low (less than 0.5), intermediate (0.5-0.99), and high (greater than or equal to 1.0) FLT3-ITD burden. Additional clinical and molecular features were retrieved from electronic medical records. Co-occurring mutations were detected by targeted next-generation sequencing of DNA extracted from bone marrow samples at diagnosis. Genomic DNA libraries were prepared and sequenced on a NextSeq 550 sequencer.

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Bioinformatic analyses were performed using in-house bioinformatic pipelines and a bandwidth-adjusted statistical method. Data on the FLT3-ITD and NPM1 mutations were retrieved from electronic medical records. For HMA primary resistance, patients were considered resistant if they had progressive disease or stable disease after 4 cycles, or did not achieve complete remission at first assessment. To validate the cohort, the analysis was performed in another independent cohort treated with azacitidine and venetoclax or targeted therapy alone. For clinical and molecular features, one or two statistically significant features were included in a chemical development cohort, depending on the complexity of the model. For FLT3-ITD burden analyzed as a continuous variable, receipt of a FLT3 inhibitor was adjusted for in the model. To validate the findings, the same covariates were used in the validating cohort. Hardy-Weinberg equilibrium was assessed for potential SNPs with P values considered statistically significant. For the cumulative effect of SNPs on FLT3-ITD burden, interaction tests and logistic regression analyses were performed. Minimal residual disease (MRD) monitoring was carried out using next-generation sequencing of FLT3-ITD alleles in bone marrow samples collected after therapy upon IRB approval. DNA libraries were prepared from genomic DNA and sequenced to a target depth of 100,000 reads per sample. To validate the assay, the sensitivity and specificity of FLT3-ITD detection were determined using in-house bioinformatic analyses and statistical methods.

FLT3-ITD Mutation Burden

The presence of FLT3-ITD mutations in acute myeloid leukemia (AML) has been associated with poor prognosis. However, the significance of FLT3-ITD allelic ratio and total mutation burden remains less clear. Recently, a novel FLT3-ITD quantitative technique was evaluated based on digital PCR technology. The prognostic value of FLT3-ITD mutation burden was assessed in a cohort of 411 patients with FLT3-ITD-positive AML, with a focus on the effects of the total mutation burden in comparison with other FLT3-ITD variants. Results indicated that the FLT3-ITD mutation burden, measured based on the ratio of total ITD mutants (mutant allelic ratio) or number of ITD copies (total ITD copy number), was an independent and robust poor prognostic factor for relapse-free survival (RFS) and overall survival (OS).

The FLT3-ITD mutation burden significantly stratified the patients into distinct prognosis groups, even in favorable-risk subgroups, such as the ATRA-induced wild-type NPM1 and CEBPA cohort. Moreover, FLT3-ITD mutation burden was superior to other anti-FLT3 TKIs therapeutics-based adjuvant strategies. Finally, it was shown that robust contrast capabilities to genotype greater than non-correlation sequence-based amplification-PCR methods were observed. The genomic position of major and minor FLT3-ITD was validated, indicating that FLT3-ITD mutations position in distinct exons correlate with rank, number, and location of different insertions. In conclusion, this study demonstrates that CLO-A4 displayed potent FLT3 inhibition, was taken up by AML cells in a concentration-dependent manner, and exhibited prolonged inhibition of cellular FLT3 phosphorylation resulting in enhanced cytotoxicity of AML

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cells, imparting a way for further exploration and development as the next-generation anti-FLT3 AML therapeutics.

Co-occurring Mutations

The presence of FLT3-ITD mutations in newly diagnosed AML is commonly associated with other co-occurring mutations. Analyzing additional mutations within an AML cohort and their relationship with FLT3-ITD mutations is critical for a comprehensive understanding of the genetic landscape of the disease. Commonly mutated genes in AML include DNMT3A, TET2, NPM1, TP53, ASXL1, and IDH1/IDH2, among others. The occurrence of co-mutations has been shown to influence the biologic behavior of the AML. Within the FLT3-ITD mutated cohort of a larger AML population, both the presence of additional mutations and the specific mutation types were found to influence overall survival (OS) and disease-free survival (DFS). FLT3-ITD mutations frequently occur in conjunction with other genes. The combined analysis of FLT3-ITD mutations and other NPM1 mutations in AML patients demonstrated that discordant mutation status is associated with a worse clinical outcome, characterized by a shorter OS and poorer response to post-remission chemotherapy.

FLT3-ITD mutations are the most common mutations in AML and frequently co-occur with NPM1 mutations. Understanding the impact of co-existing mutations on treatment response and clinical outcomes can better inform the risk stratification of AML patients. Once thought to be largely random, co-mutated genes appear to exist within networks in AML. An interactive web-based tool is created for exploring the detected genes co-occurring with FLT3-ITD and NPM1 mutations in AML. Simultaneous mutations of FLT3 and NPM1 indicate genetic interaction via a mutual pathway. Network parameters of the genes co-occurring with genetic mutations were significantly correlated with mRNA expression levels, highlighting the role of genetic mutations in shaping the landscape of expression co-regulation in AML. The study finds that nine genes co-occurring with FLT3-ITD are involved in hematopoiesis and cell-cell adhesive ligands. Loss of CFI induces a hyperproliferative phenotype and contributes to a higher risk of the "poor" prognostic category in FLT3-ITD+ AML patients after chemotherapy.

Minimal Residual Disease Monitoring

Comprehensive characterization of residual disease is paramount in FLT3-ITD-positive AML, and optimized methodologies have been introduced for it. Nevertheless, accurate quantification of MRD in FLT3-ITD-positive AML samples is crucial for patient outcome predictions. As FLT3-ITD mutations are highly heterogeneous and variable, extensive attention is needed for their accurate quantification. The employment of DNA-immunoprecipitation and earPCR methodologies allows for allele ratio normalization, increasing the reliability of FLT3-ITD detection. Investigating the feasibility of MRD monitoring in FLT3-ITD-positive AML using constant-detectable-attanges approaches may better address this concern.

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Caution is needed when interpreting studies utilizing MRD quantification in non-ITD FLT3 mutations, as in some studies, investigations were confined to the presence or absence of FLT3 mutations without differentiating between ITD and TKD subtypes. It is unexpected that very few relevant studies focusing on FLT3-ITD AML have been published in the post-allo SCT setting. The fact that FLT3-ITD-positive subtype is frequently not analyzed by MRD assessment in any of the published analyses may be attributable to the relatively low sample sizes of FLT3-ITD-positive patients in these studies. Further examinations regarding the impact of FLT3 mutation status, co-mutations, and allele ratios on MRD monitoring in these cohort analyses may refine MRD analyses in FLT3-ITD-positive AML and investigation of novel mutation targets beforehand can avoid confounding effects on MRD assessment results. Beyond dPCR methods outlined here, newly developed approaches like targeted GeneScan methodology for variant-specific detection of FLT3-ITD mutations broaden the feasibility of MRD monitoring in FLT3-ITD-positive AML.

Mechanisms of Relapse and Refractory Disease

The field of acute myeloid leukaemia (AML) has witnessed the emergence of novel genetic aberrations related to the disease pathogenesis. However, the majority of patients eventually relapse following intensive therapy, and a large number are refractory to initial therapy. The prognostic relevance of these mutations with respect to analyzing novel mechanisms of relapse and refractory disease is focused in this section. FLT3-internal tandem duplication mutations (ITDs) arise early during the course of disease initiation and progression. Their persistence during and after therapy has been associated with a poor clinical outcome. Nevertheless, the mechanisms of treatment response, resistance, and disease recurrence of FLT3-ITD have not been thoroughly investigated. As suggested by a number of studies on various oncogenic lesions, clonal evolution seems plausible in FLT3-mediated leukemogenesis. Several databases deposited a comprehensive catalogue of secondary mutations and copy number alterations (CNAs) found in 81 matched diagnosis-relapse AML pairs. It is noteworthy to track the accumulation of 15 recurrent gain-of-function mutations clustering in a hotspot at the Phospho-tyrosine-binding domain of the superoxide dismutase 1 (SOD1) gene across disease progression. This network further predicted increased oxidative stress resulting from aberrant SOD1 activity as a potential mechanism of FLT3-ITD-related leukemogenesis. To provide a valuable resource for the field, an analytic review of the potential mechanisms of relapse and refractory disease in FLT3-ITD AML based on clonal evolution and targeted pseudo-correlation is given. The clonal evolution of FLT3-ITD AML upon treatment is investigated by an integrative analysis of exome sequencing data from AML patients at diagnosis and relapse, together with their matching germline samples. This analysis identifies novel recurrent mutations in genes including SOD1 that play a role in FLT3-ITD-related leukemogenesis. Further functional studies are proposed to examine cooperation among FLT3-ITD, TET2 and SOD1 mutations, as well as to investigate whether the MASA transcriptional network functions as a driver converting pre-

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leukemic states to overt leukemia. The FLT3-ITD-induced transcriptional dysregulation, potential SOD1 downstream targets and mechanisms of transcriptional alteration will be systematically delineated. On the other hand, the interaction of FLT3-ITD hematopoietic stem cells with normal niche cells in the remodeling microenvironment is explored. The role of CCL-5/CCR5 interaction within the AML niche in mediating the resistance of leukemia cells to chemotherapy is also investigated. Considering the limited understanding of clinically relevant mechanisms of relapse and refractory disease in FLT3-ITD-positive AML, it is proposed to develop a more effective disease model and necessary resources for the AML research community to dissect novel molecular mechanisms underlying cancer relapse and therapeutic resistance, as well as for potentially better therapeutic solutions.

Clonal Evolution

Relapse after achieving first complete remission (CR1) is a major concern in acute myeloid leukemia (AML), both for therapy-related and de novo cases. Overall survival rates in FLT3-ITD-positive AML remain poor despite modern standard of care, and patients who relapse or are refractory to FLT3 inhibitors have limited treatment options. This highlights the need to understand the underlying mechanisms of FLT3-ITD-positive AML clonal evolution and resistance, to facilitate the design of better strategies modeling resistance in vitro, altering this process before, during, and after therapy, as well as the development of combination therapeutic strategies in patients with FLT3-ITD-positive AML.

Large-scale sequencing studies have established that the mutational landscapes of AML change as therapy is given, with the emergence of either new mutations or the progressive increase in variant allele fraction (VAF) of existing mutations. This is consistent with the clonal evolution model of cancer, which describes the process of a malignant tumor increasing in genetic aberrations and heterogeneity over time. Beyond VAF-based analysis, draFLIP was developed to obtain an in-depth view of individual clones across different time-points. This approach is based on the fact that recombination events propagate in the same clone and are preserved even after many cell divisions. Another approach found that the number and type of somatic mutations were preserved between the paired diagnosis and relapse samples. This approach required only low-pass WGS data, highlighting its applicability for further studies using vaginal swab samples.

The consequences of clonal evolution have been investigated in diverse cancers. Analysis of mismatched control vs. treatment-exposed relapses revealed a large continuous emergence of new variants during therapy and a high mutation burden in the resistant clones. This study proposed that continued exposure of the cancer cells to the same targeted therapeutics might permit the development of resistant clones encoding a larger number of mutations, as these mutations could afford survival benefits that counterbalance deleterious side effects. CLIP6 and CDKN2A/B mutations were associated with rapid subsequent relapse after FLT3 inhibition in

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patients with FLT3-ITD-positive AML. Experimental models of FLT3-ITD and CLIP6 dual mutations identified a putative mechanism of oncogene cooperation that circumvents FLT3 inhibition resistance. Systematic analysis of the clonal evolution of NPM1 mutant leukemias through serial transplantations in mice revealed that most clonal evolution occurred during the formation of the first recipient primary engraftment. NPM1 mutant clones proliferated poorly in a latent state until their epigenetic state was altered in the recipient environment, resulting in a sudden re-entry into the cell cycle.

Microenvironment Interactions

The tumor microenvironment plays a crucial role in leukemogenesis and development of malignancy in antileukemic therapy. Tumor microenvironment can confer drug resistance on malignant cells via production of soluble factors such as cytokines and chemokines, interactions with extracellular matrix (ECM) proteins, and direct cell-to-cell interactions. FLT3-ITD mediates diverse interactions with microenvironment components such as monocytes and endothelial cells, and confers distinct cytokine/growth factor secretion profiles. These interactions contribute to pharmacological resistance to cytarabine and FLT3 inhibitors in FLT3-ITD-positive acute myeloid leukemia (AML) cells and enhance AML cell migration. Aberrant production of IL-1 family members up-regulate in FLT3-ITD-positive AML cells after exposure to endothelial cells and monocytes. Excessive and aberrant IL-1β, IL-1α, and IL-36γ in FLT3-ITD-positive AML support disease progression. Several investigations have focused on the identification of novel therapeutic targets to overcome drug resistance mediated by the tumor microenvironment in FLT3-ITD-positive AML. The aberrant tumor microenvironment contributes to pharmacological resistance via diverse mechanisms. A paracrine loop between FLT3-ITD-positive AML cells and microenvironmental components that aggravates drug resistance and promotes the aggressiveness of FLT3-ITD-positive AML has been identified. Engaging these unique interactions could be useful to develop novel therapeutic strategies to target the tumor microenvironment for FLT3-ITD-positive AML.

Predictive Models and Prognostic Factors

Although great progress has been achieved in the study of FLT3-mutated AML, many questions remain open, particularly regarding the gross neglect of FLT3-ITD/M in younger patients and candidate drugs for clinical tests persist in failing. There is still no approved MRD assay, clinical trial, or drug target for FLT3-M, and the possibility of aborting treatment in cases with low FLT3 levels. Additionally, FLT3 is considered a high-risk abnormality due to poor prognosis, but this overlooks certain low-risk cases whose complete remission rates rival those of NPM1 and CEBPA, two individuals associated with excellent prognosis. Furthermore, concerning the morphologic spectrum of FLT3-M, ongoing rapid evolution toward monoblastic and myelodysplastic MDS subtypes is observed.

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Predictive models to estimate relapse and refractory risks in FLT3-ITD-positive AML cases have been developed using clinical and multigenetic factors. Factors identified as independent prognostic variables are: age, WBC count, cytogenetic risk, type of FLT3-ITD mutation, co-occurrence of NPM1 mutation, and WT1 expression pattern. Systematic model derivations were performed in the entire cohort and after confining analysis to a particular WBC count range. These models constitute useful tools to estimate relapse and refractory probabilities, as both risks may crosstalk in individual cases.

Genetic Risk Stratification

Genetic risk stratification is commonly used to guide treatment in different subgroups of ADE patients; however, concerns were expressed about the neglect of the FLT3-ITD mutation, the most common driver mutation along with NPM1. The role of the FLT3 mutation in the diagnosis was investigated in ~10,000 cases of primary adult ADE, RNA-seq-based genetic risk stratification, and clinical data curated from the blood-cancer genome atlas cohort. The FLT3 mutation was also examined in a broader cohort (~20,000 cases) of ongoing transcriptomic risk stratification initiatives.

Results confirmed that FLT3-ITD is the most prevalent mutation (~26%) and elucidated discrepancies between clinical and molecular risk stratification. FLT3-ITD cases were significantly enriched in the high-risk cohort, but a considerable number of FLT3-ITD cases were clustered with the intermediate- and low-risk cohorts or exclusively harbored favorable NPM1 or CEBPA mutations. Inverse results were consistently observed within the context of chemotherapy regimens.

In contrast to Genomic Organizations (GOF) genes, equal vigilance should also be placed on loss of function mutations (LOF), as both are embedded in distinct stratification schemes. The NPM1 mutation is frequently associated with other LOF mutations in the splicing factor SRSF2 or epigenetic modulator TET2.

Novel Biomarkers

Novel biomarkers were sought to better stratify FLT3-ITD-positive patients. Several RNA-seq-derived gene-expression profiles showed negative correlations with remission probabilities in the first cycle of intensive therapy with cytarabine and anthracyclines, as they constitute informative markers of overall treatment response. The analysis was then narrowed down to a single continuous variable to estimate FLT3-ITD identity. After controlling for both confounding variables of WBC count and age, several multivariate models could estimate FLT3-ITD size ratio and the number of ITD loci. However, going beyond technical means, it forgoes unmet biological questions on target recruitment and the mechanism of action.

Genetic Risk Stratification

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Flt3-ITD (Fms-like tyrosine kinase 3-internal tandem duplication) is one of the most common mutations observed in acute myeloid leukemia (AML), carrying a poor prognosis due to high rates of early relapse, especially in patients aged <60 receiving "7+3" chemotherapy. A comprehensive multivariable analysis was done for Flt3-ITD positive AML to evaluate the effect of age, sex, cytogenetics, and several gene mutations on disease-free survival. This analysis attempts to identify key risk factors to better simplify the problem of risk stratification in this group of patients. Flt3-ITD AML was focused on and well-studied as it tended to be high-throughput in technology. Reliable multivariable models of Flt3-ITD AML have not been explored independent from common AML analysis mainly due to the difficulty of extracting specific subtypes from large databases. Analyzed variables consisted of 1591 unique Flt3-ITD AML samples with 12 genomic mutations and 120 classically-defined cytogenetic abnormalities. A total of 33 multivariable random forest models were built and analyzed in relation to discriminatory ability, variable importance, and parsimoniousness, evaluating the significance of 14 candidate factors balanced for 6 events per variable. Detected factors were organized into four groups: Basic (age and sex), Cytogenetic (12 frequently-observed abnormalities), Genomic (7 mutation types), and Aggregate (6 combined classes). These analyses reconfirmed the canonical knowledge of good, moderate, and poor genetic risk groups in AML based on predictive variables like age, cytogenetics, and Flt3-TKD. This showed how aggregation of various negative mutations could further stratify the risk of relapse even in some of the widely-known groups so far. High-throughput genomic annotations were observed increasingly used for extraction of many more features like CNV or expression levels but has yet to be extensively explored in AML. High-throughput analysis has the potential to transform the understanding of both normal biology and disease pathophysiology through the identification of underlying key drivers in prediction of treatment response and risk stratification.

Novel Biomarkers

Novel biomarkers measured prior to treatment have been hypothesized as additional prognostic factors in FLT3-ITD-AML. Several of these biomarkers have been measured as part of clinical trials enrolled and completed prior to the widespread use of FLT3 inhibitors. Considering only studies featuring OOC, a total of 339 studies across 89 publications were identified. Several novel biomarkers, including ALT-PE, MPO-9, CCL2, CCL3, plasma cell-free DNA (cfDNA), EDN1, HMGB1, and MX1, have been identified in ≤ 3 publications analyzing ≥ 10 patients.

The current body of literature regarding how these novel biomarkers have been studied, and subsequently their prognostic impact in FLT3-ITD-AML, is as follows: (i) the prognostic impact of elevated levels of CCL2 demonstrated significance in a multi-center study including 127 patients; (ii) the prognostic impact of elevated levels of CCL3 demonstrated significance in a single-center discovery and validation approach including 94 patients; (iii) the prognostic impact

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of elevated levels of CCL3/CCL2 demonstrated significance in a multi-center study including 282 patients; (iv) the prognostic impact of low levels of EDN1 upon group analysis of 76 patients with FLT3-ITD demonstrated significance in a two-hit model approach; (v) the prognostic impact of an elevated cfDNA concentration having a concordant increase of the cfDNA-ITD demonstrated significance in a multi-center analysis including nosologically heterogeneous AML patients; (vi) the prognostic impact of low levels of HMGB1 demonstrated significance in a multi-center validation study of 60 patients; (vii) the prognostic impact of elevated levels of MX1 demonstrated significance in a multi-center discovery and validation approach including 88 patients; (viii) the comparison of results in patients that received only intensive chemotherapy vs. salvage chemotherapy receiving RIC. In summary, there are many identified prognostic biomarkers beyond the currently accepted canonical mutations. Additional multi-cohort studies should be carried out to determine their generalizability. As the FLT3 inhibitors continue to be integrated into frontline therapy, it will be important to reassess the prognostic impact of these novel biomarkers in the context of MRD monitoring.

Therapeutic Developments

The risk of relapse remains high for FLT3-ITD-positive acute myeloid leukemia (AML). However, targeted therapies for these mutations, whether examined as single agents or in combination with chemotherapy, are now being developed. Immunotherapies targeting FLT3-ITD and ongoing clinical trials of exciting agents such as asparaginase and venetoclax are also discussed. Pre-clinical models that accurately review the relapse and refractory risks with the designed models for FLT3-ITD AML are also described. The internal tandem duplication (ITD) of the FMS-related tyrosine kinase 3 (FLT3) receptor tyrosine kinase is one of the most common mutations in acute myeloid leukemia (AML). FLT3 mutations are detected in 20-30% of newly diagnosed AML patients, and those mutations correlate with a poor prognosis. High allelic ratio (AR) FLT3-ITD is reported as a poor prognostic factor when considered alone as compared to normal cytogenetics. Mutations in the FLT3 gene, commonly an internal tandem duplication (FLT3-ITD) or a point mutation in the tyrosine kinase domain (TKD), are among the most common mutations in AML and are associated with poor clinical outcomes. The development of novel therapies targeting FLT3 mutations has led to the emergence of FLT3 inhibitors (either type I or type II). Type II inhibitors selectively bind to the inactive conformation of the mutant FLT3 kinase and retain activity against the FLT3-ITD mutations. FLT3 inhibitors exhibit better survival in FLT3-ITD patients after first relapse and are currently being investigated in post-remission strategies for FLT3-ITD AML patients. FLT3 inhibitors at relapse result in a low response rate within a median of 24 weeks. Relapse usually occurs with the constitutive reactivation of FLT3 signaling related to secondary mutations in FLT3 or loss of expression of type II FLT3. Comparison of pre-clinical and clinical data on the risk of relapse and resistance mechanisms may be useful to develop therapeutic strategies that eliminate or delay resistance to type II FLT3 inhibitors.

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The formulations of monoclonal antibodies targeting a peptide derived from a FLT3-ITD consensus sequence are under investigation in a phase 1 study. Furthermore, the combination of FLT3 inhibitors with vaccines has demonstrated improved survival in pre-clinical models and is moving into the clinical space. Continued research to improve outcomes for this high-risk patient population is warranted. FLT3-ITD is among the most common mutations seen in AML and is classified into three types based on length and AR. High-AR FLT3-ITD mutation tends to have worse prognosis compared to low-AR FLT3-ITD mutation when examined independently of other factors or in the context of currently standard 7+3 chemotherapy regimen. However, this observation does not hold true for other regimens, such as 3+7 and FLAG-IDA, or in the contexts of post-chemotherapy settings indicating that further studies are needed to elucidate the prognosis of FLT3-ITD in differential chemotherapy context. In addition, there remain unanswered questions regarding the characterization of AML samples with concurrent mutations.

Emerging Targeted Therapies

The continued understanding of FLT3-ITD driven leukemogenesis has allowed researchers to efficiently develop drugs targeted on the mutant receptor. Harnessing the intrinsic properties of tyrosine kinases, an array of small molecules designed to inhibit dimerization and kinase activity have been brought into preclinical and clinical development with the hope of impeding disease progression. While a number of agents have been developed to target FLT3-ITD signaling, there are still many issues that remain before targeted therapy can be considered standard of care. Drug development has accelerated with the entry of a number of small molecule inhibitors of FLT3 into clinical trials following the initial reports of efficacy from sorafenib in 2005 and the multi-kinase inhibitor, lestaurtinib (KT-6588), in 2006. Both early trials demonstrated that FLT3 mutations are capable of inducing a more aggressive clinical disease and can also alter the overall safety profile of conventional chemotherapy. Early treatment with a FLT3 inhibitor post-remission can prolong relapse-free periods when compared to chemotherapy alone and may be optimal when combined with allogeneic stem cell transplant. Despite several rigorous attempts at large scale testing of FLT3 isolates in both patient cohorts and preclinical models, no single candidate FLT3 inhibitor has yet gained widespread clinical indication or FDA approval. Novel agents are needed to overcome the challenge of secondary mutation acquisition in patients already treated with a prior FLT3/ITD-kinase inhibitor. Beyond the problem of resistance mutations, the vast majority of patients fail to respond to initial therapy with FLT3 inhibitors due to high levels of innate cytosolic drug resistance. Though it is clear that FLT3 inhibitors will become a major focus of therapy in younger patients, the exact timing of transition from being used in initial post-remission consolidation to front-line treatment in cytogenetic strata with poor risk has yet to be clearly defined. It will be extremely difficult for FLT3 inhibitors to effectively combat disease sporadically co-harboring multiple receptor tyrosine kinases without the discovery of pan-tyrosine kinase inhibitors.

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Immunotherapeutic Approaches

Recent decades have witnessed the emergence of various innovative immunotherapeutic approaches, including monoclonal antibodies and monoclonal antibody-drug conjugates, CAR-T cells, (bispecific) T cell engagers, DNA vaccines, and immune checkpoint inhibitors. A number of precursor studies demonstrating FLT3-ITD-specific monoclonal antibodies, α-FLT3 ITD, capable of recognizing Golgi-localized forms of the mutant receptor have been performed. Preclinical testing of α-FLT3 ITD reinitiated a full cohort of MLL-AF9 FLT3-ITD mice on a dose-escalation trial with an emphasis on the final most efficacious dose of 75 µg. At this, both peripheral blood counts were markedly improved and FLT3-ITD positive leukemic splenomegaly was significantly reduced, differentiating the trial cohort from untreated FLT3-ITD positive MLL-AF9 controls. Apoptotic effects of α-FLT3 ITD were further demonstrated ex vivo in mononuclear splenocytes with a significantly increased proportion of apoptotic leukemic blasts at 48 hours post-treatment, isolated from the previously monitored leukemia-engrafted MLL-AF9 FLT3-ITD mouse cohort. Overall, these findings suggest that durable eradication of FLT3-ITD leukemia through the activation of the humoral immune system may be feasible with appropriate dose and schedule optimization.

Gene-modified T cell therapy for FLT3-ITD positive AML has also been shown to be feasible in preclinical experiments. AML blasts with high expression levels of FLT3-ITD showed robust T cell activation, and activation and proliferation of gene-modified T cells was observed early after co-culture. Clearance of engrafted FLT3-ITD positive AML was accompanied by a significant reduction in bone marrow infiltration of AML blasts and a reduction in splenomegaly. Furthermore, systemic office-convalescent E4 passaged MLL-AF9 FLT3-ITD leukemia, established in 5 C57Bl/6 mice using the co-injection approach and confirmed through RT-PCR assays for the presence of FLT3-ITD, the NMP and vH4-IgG fluorescent probes were used to quantify normal and transformed NFxN-AP-1 levels in hematopoietic stem and progenitor cells of treated and untreated mice. A targeted therapy approach for FLT3-ITD positive AML has been shown to break the oncogenic cooperativity and stimulate tumor development through the formation of west target gene modules, ultimately leading to tumor relapse.

Conclusion

Acute myeloid leukemia (AML) is a group of heterogeneous hematologic malignancies characterized by the clonal expansion of myeloid progenitor cells. In adults, FLT3-ITD mutations are among the most common genetic alterations in AML, occurring in 20-30% of patients, with adverse prognostic implications. After intensive induction chemotherapy with cytarabine and an anthracycline, approximately two-thirds of patients with FLT3-ITD positive AML will experience relapse. In this review, the spectrum of FLT3-ITD positive AML with an emphasis on the causes and potential mechanisms of resistance to standard therapies are discussed, with a focus on potential strategies to mitigate the risk of treatment failure.

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FLT3 is a membrane-bound receptor tyrosine kinase that regulates hematopoiesis and plays a pivotal role in FLT3-ITD positive AML. The mutated FLT3 receptor becomes constitutively active, transmitting growth and survival signals to downstream pathways regardless of ligand activation. Prior to treatment, the size of the FLT3-ITD DNA subclone relative to the wild type is an important predictor of risk of death after relapse. Following standard chemotherapy, the proportion of FLT3-ITD burden loss events within a duration of approximately 35 days after therapy appears to underpin improved overall survival in FLT3-ITD patients, suggesting that FLT3-ITD positive clones may be inherently more vulnerable to S-phase-targeted agents.

The effects of FLT3-ITD in AML can depend on numerous factors that can alter the resultant downstream signaling. These include the mutation's length (i.e. number of base pairs that have been inserted), localization of the mutation in the FLT3 receptor (internal vs. terminal), presence of co-occurring mutations, activation status of upstream pathways that lead to FLT3 phosphorylation, and differing expression levels of various scaffold and regulatory proteins. These factors can all combine to influence the availability, activation, or interactions of FLT3 and its downstream components. Depending on these alterations, FLT3-ITD in AML can provide activating signals that are weak and transient in nature, or could sustain aberrant downstream signaling despite chemical inhibitors or off-target effects of other therapies.

A small subset of children with NPM1 or MLLT10-FLT3-ITD positive AML may have an observable interleukin 1 beta (IL1B) cytokine expression signature. This signature is present in FLT3-ITD positive pediatric AML with aberrant Hoechst 33342 dye efflux. FLT3-ITD positive AML with high IL1B expression was found to have poor prognosis and low overall survival rates. The IL1B expression signature conferred FLT3 independent signaling downstream of the CCL2 receptor. Its use as a therapeutic target is complicated by the ubiquitous presence of inflammatory signals, which could engender unwanted on-target off-tumor effects, as well as potential resistance mechanisms that arise from signaling crosstalk.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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