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FLT3, General AML

# Investigating Copy Neutral Loss of Heterozygosity (CN-LOH) in FLT3 AML Patients: A British Study

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Adult Acute Myeloid Leukemia (AML) that presents with a Normal Karyotype (NK-AML) are characterised with frequent FMS-like Tyrosine Kinase 3 (FLT3) mutations. FLT3 with internal tandem duplication (FLT3-ITD) predicts a more aggressive disease associated with resistance to therapy and poor survival in this subgroup of patients.

Moreover, Raghavan *et al*[1] reported that chromosomal defects such as Copy Neutral Loss of Heterozygosity (CN-LOH) can arise in AML, causing patients to lose the Wild Type (WT) FLT3 allele and acquire the mutated FLT3-ITD allele.

Acquired CN-LOH in AML can lead to resistance to standard therapy and result in poor survival outcomes in this patient group. The etiology underlying the development of acquired CN-LOH remains unknown, although it is thought that chromosomal homologous recombination (iHR) activity maybe involved and hence the rationale for this study.

Terry J Gaymes from Kingston University, United Kingdom, and colleagues published the results from their study in Cancer Research in January 2017.

The authors investigated whether there was an association between constitutive FLT3 and JAK2 signaling and the propagation of CN-LOH in cells from 22 *de novo* AML patients (median age = 53 years). Using a novel recombination assay, the authors aimed to detect Homologous recombination (HR) events in mutated FLT3 and JAK2 cells.

**The key results are:**

- iHR events for MOLM-13 (*FLT3-ITD* expressing cells) and *FLT3-ITD* transfected HEK293PZD cells were 0.15% and 0.25% respectively compared to 0% in WT FLT3 expressing cells; $P < 0.05$, n = 3
- AC220 (FLT3 inhibitor) pre-treatment downregulated RAD51 expression, a measure of HR, and abolished iHR events in *FLT3-ITD* transfected HEK293PZD cells and MOLM-13PZD
- Significantly increased HR activity in primary FLT3-ITD (n = 4) compared to primary WT FLT3 (n = 2); 1.2% *vs* 0.25%, $P < 0.01$
- Pre-treatment of primary cells with AC220 or N-acetyl cysteine (NAC) (an anti-oxidant) significantly inhibited HR activity; 1.2% *vs* 0.1%, $P < 0.01$
- Significant elevation of reactive oxygen species (ROS) in JAK2V617F cells
- Significant elevation of spontaneous thymidine kinase (TK) mutation rate and frequency in *FLT3-ITD* compared to WT *FLT3*
- AC220 and NAC significantly inhibited CN-LOH frequency at TK locus in *FLT3-ITD* cells and WT FLT3 cells

In summary, the findings of study revealed that mutated FLT3-ITD and JAK2 augment reactive oxygen species (ROS) production and HR thus leading to iHR and the subsequent acquisition of CN-LOH in NK-AML. Treatment of FLT3-ITD and JAK2 mutated cells with NAC, decreased ROS. Additionally, common breakpoints in the TK locus contributes to the propagation of CN-LOH.

Furthermore, the authors suggests that anti-oxidants could be used alone or in combination with conventional therapies to prevent accumulations of mutations and the subsequent acquisition of CN-LOH in order to slow the rate of disease progression in NK-AML. The authors concluded by stating that novel therapies targeting the reduction of ROS would be of clinical importance for the treatment of patients with FLT3 and JAK2 mutations.

**Abstract**

Acquired copy neutral loss-of-heterozygosity (CN-LOH) is a frequent occurrence in myeloid malignancies and is often associated with resistance to standard therapeutic modalities and poor survival. Here we show that constitutive signaling driven by mutated FLT3 and JAK2 confers inter-chromosomal homologous recombination (iHR), a precedent for CN-LOH. Using a targeted recombination assay, we determined significant iHR activity in internal tandem duplication FLT3 (FLT3-ITD) and JAK2V617F mutated cells. Sister chromatid exchanges, a surrogate measure of iHR, was significantly elevated in primary FLT3-ITD normal karyotype acute myeloid leukemia (NK-AML) compared to wild type FLT3 NK-AML. Homologous recombination (HR) was harmonized to S phase of the cell cycle to repair broken chromatids and prevent iHR. Increased HR activity in G0 arrested primary FLT3-ITD NK-AML in contrast to wild-type FLT3 NK-AML. Cells expressing mutated FLT3-ITD demonstrated a relative increase in mutation frequency as detected by thymidine kinase (TK) gene mutation assay. Moreover, resistance was associated with CN-LOH at the TK locus. Treatment of FLT3-ITD and JAK2V617F mutant cells with the antioxidant N-acetylcysteine diminished reactive oxygen species (ROS), restoring iHR and HR levels. Our findings show that mutated FLT3-ITD and JAK2 augment ROS production and HR, shifting the cellular milieu towards illegitimate recombination events such as iHR and CN-LOH. Therapeutic reduction of ROS may thus prevent leukemic progression and relapse in myeloid malignancies.

**References**

1. Raghavan M.*et al.*, Segmental uniparental disomy is a commonly acquired genetic event in relapsed acute myeloid leukemia. Blood. 2008; 112:814-21
2. Gaymes T.J. *et al.*, FLT3 and JAK2 mutations in acute myeloid leukemia promote inter-chromosomal homologous recombination and the potential for copy neutral loss of heterozygosity (CN-LOH). Epub 2017 Jan 20. Cancer Research. DOI: 10.1158/0008-5472.CAN-16-1678.