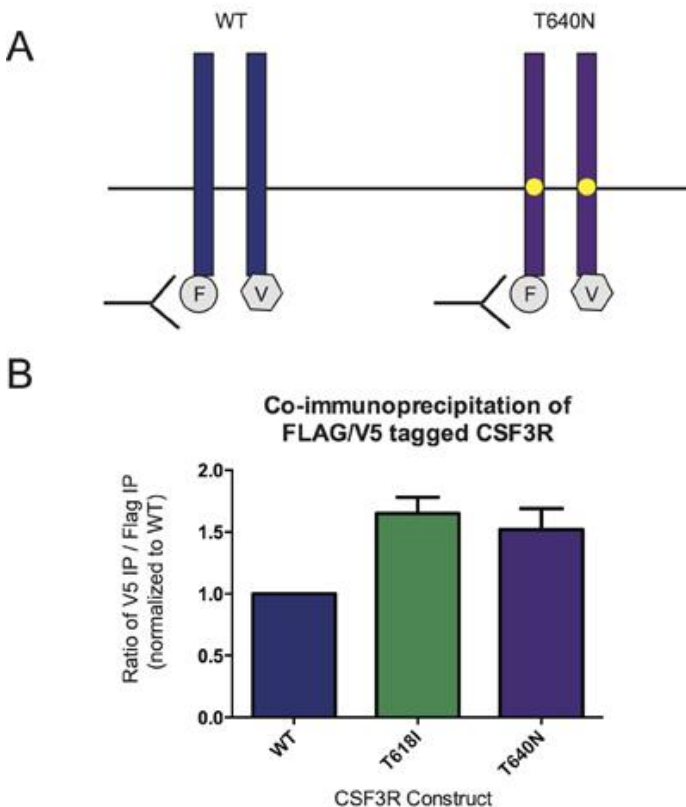


No stone unturned: rare mutation in CSF3R may inform therapy

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T604N mutation increases CSF3R dimerization (A) Schematic of co-immunoprecipitation experiment to quantitate CSF3R dimerization. (B) The T640N mutation has increased dimerization relative to WT CSF3R. Flag and V5-tagged CSF3R constructs are co-transfected into cells, then an antibody to flag is used to immunoprecipitate CSF3R complexes. Co-immunoprecipitated V5 tagged CSF3R is detected by immunoblotting and is normalized to the input.

Image provided by Dr. Julia Maxson (Clinical Research Division).

Cancer is the second leading cause of death in the U.S.A. with over 1.5 million individuals being diagnosed every year. While cancer is often discussed as a single disease, it is an expansive and diverse set of diseases each requiring a unique treatment. In the face of these staggering numbers of patients and pathologies, rare cancers are often overlooked due to challenges in funding. Recruiting sufficient numbers of patient samples for clinical trials presents another challenge. Chronic neutrophilic leukemia (CNL) and atypical chronic myeloid leukemia (aCML) are two such rare cancers; they are characterized by high levels of neutrophils or neutrophil precursors and lack molecular signatures seen in most CML patients, such as BCR-ABL1 fusion, or rearrangements of PDGFR or FGFR. Instead, recent studies have identified colony stimulating factor 3 receptor (CSF3R) as a common oncogenic driver in CNL affecting approximately 80% of patients. In these

patients the most common mutation is a substitution, T618I, which is sufficient to drive oncogenesis in multiple in vitro models. This mutation causes ligand-independent dimerization of CSF3R and results in constitutive signal transduction through the JAK pathway. CSF3R bearing the T618I mutation is also hypo-glycosylated, however, the relationship between glycosylation and dimerization remains unknown.

In a recent study published in *Clinical Cancer Research*, Dr. Julia Maxson, a postdoctoral fellow in the Radich Laboratory (Clinical Research Division), identified a CSF3R mutation that has never been observed in CNL and aCML patients. Sequencing the CSF3R gene in two patient samples revealed the mutation, T640N, which was first identified in rare cases of AML. While this study focused on findings in CNL and aCML, Dr. Maxson has observed this mutation in other cancer types, "I'm also collaborating with Soheil Meshinchi on a project looking at CSF3R mutations in pediatric AML. Interestingly, the CSF3R T640N mutation was also found in two of these patients." In the current study the authors demonstrated that the T640N conferred many oncogenic behaviors to hematopoietic cells including the ability to grow independent of cytokine stimulation, and colony formation independent of the CSF3R ligand. These results suggested that T640N was acting through the same mechanism as the common T618I mutation – ligand independent dimerization. Molecular modeling from a previous publication predicted the T640N mutation would drive dimerization through increased hydrogen bonding. As predicted, the mutation increased ligand-independent receptor dimerization, however, this unique mutation provided a novel insight into the relationship of glycosylation and dimerization. T640N, unlike T618I, resides in the transmembrane region of CSF3R, which should preclude any physical interaction with glycosyltransferase enzymes. Interestingly, just like the common T618I mutation, T640N also decreased total levels of glycosylation on CSF3R. These findings raise the possibility that decreased glycosylation is a result of increased receptor dimerization, where glycosyltransferases are sterically occluded from the residues they normally act on.

These researchers verified the oncogenic nature of the T640N mutation in a mouse leukemia model, demonstrating that at the pathological level it behaved equivalent to the T618I mutation. Cells harboring the T618I mutation in CSF3R are known to activate the Janus Kinase (JAK) protein family, and these cells demonstrate sensitivity to JAK inhibitors. Excitingly, the authors find that the T640N mutation induces a greater sensitivity to this class of inhibitors. This work makes a strong case for using both mutations as biomarkers to identify patients who will respond well to JAK inhibitors, however, such clinical studies have not yet been performed.

The use of personalized genetic analysis to improve treatment options is a common theme in Dr. Maxson's research. In a soon to be published article in *Cancer Research*, she uses these approaches to identify new therapeutic targets for leukemia and expand those findings into solid tumors. This work is among a new class, demonstrating that personalized medicine cannot rely only on genomic and expression data sets, but must also be approached with a functional perspective.

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[Maxson JE, Luty SB, MacManiman JD, Paik JC, Gotlib J, Greenberg P, Bahamadi S, Savage SL, Abel ML, Eide CA, Loriaux MM, Stevens EA, Tyner JW](#). 2015. The colony stimulating factor 3 receptor T640N mutation is oncogenic, sensitive to JAK inhibition, and mimics T618I. *Clin Cancer Res*. doi: 10.1158/1078-0432.CCR-14-3100. [Epub ahead of print.]