FGFR3 mutations are an adverse prognostic factor in patients with t(4;14)(p16;q32) multiple myeloma

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Abstract

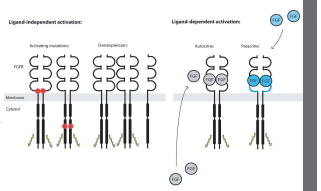
Multiple Myeloma is a hematological malignancy of terminally dfferentiated post-germinal center B-cells (plasma cells) genetically characterized by recurrent aneuploidy patterns and translocations. One common translocation is t(4;14)(p16.3;q32.2), which occurs in ~13% of patients and results in overexpression of *FGFR3* and *WHSC1/MMSET/NSD2*. We previously found that all t(4;14) tumors dysregulated *WHSC1*, however only 75% express *FGFR3*, suggesting that *FGFR3* is not the primary target of this structural rearrangement. Studies have reported known activating mutations in *FGFR3* in 5-10% of t(4;14) tumors and the lack of prognostic significance in t(4;14) based on FGFR3 expression. *FGFR3* mutations have been linked with the constitutive activation of downstream signaling pathways such as PI3K, mTOR, RAF, RAS, MAPK, and STAT which has led to clinical trials of FGFR3 inhibitors. Here, we show the first indication of adverse clinical prognosis of t(4;14) patients overexpressing mutated *FGFR3* isoforms.

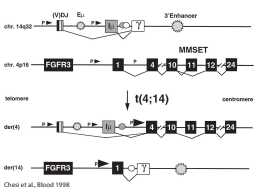
As part of the interim analysis 11 of the MMRF CoMMpass trial (NCT145429), patients with clinical data, Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES), and RNA sequencing (RNAseq) assays performed at diagnosis were analyzed regarding FGFR3 mutation, expression, t(4;14) status, and clinical outcome. Analysis of the WGS dataset for translocations between an immunoglobulin loci and 4p16 identifed classic t(4;14) events in 13% of patients (109/850), plus one translocation involving the kappa locus and none with the lambda locus. Besides these events, we identified non-immunoglobulin translocations creating novel fusion genes between *WHSC1* and *SUB1*, *HSP90B1*, *FUT8*, *CREB3L2*, or *CXCR4*. Receiver operator characteristic (ROC) analysis defined the percent of t(4;14) patients with FGFR3 expression as 72% (59/82). Confirming previous reports, survival analysis yielded a nonsignificant ($p = 0.7$) association of survival for t(4;14) patients based on FGFR3 expression. WES detected non-synonymous *FGFR3* mutations in 23% of t(4;14) patients (25/107) compared to previous reports of only 10%. *FGFR3* mutations were only observed in FGFR3-expressing t(4;14) patients and not all *FGFR3* mutated patients express pure mutated isoforms, suggesting the mutations, at least in some patients, are late events occurring after the translocation. Additionally, non-synonymous WHSC1 mutations were observed in five patients: three in FGFR3-expressing t(4;14)$^+$ and two in t(4;14)$^-$, with one t(4;14)$^+$ patient harboring both FGFR3 and WHSC1 mutations. We next investigated the correlation of *FGFR3* mutations, expression and survival within t(4;14) by stratifying 506 patients with all data types into four categories: t(4;14)$^+$ with expressed FGFR3^{mut} (n = 12), t(4;14)$^+$ with expressed FGFR3^{wt} (n = 29), t(4;14)$^+$ without FGFR3 expressed (n = 16), and t(4;14)$^-$ patients (n = 449). Our analysis shows a statistically significant ($p = 0.02$) correlation of adverse prognosis in t(4;14)$^+$ FGFR3^{mut} expressing patients (median survival = 2.8 years) compared to t(4;14)$^+$ FGFR3^{wt} expressing patients (median survival not reached).

The detection of six non-classical translocation events, all targeting *WHSC1* and not *FGFR3*, provide additional genetic evidence that *WHSC1* is the target of t(4;14). Although not the primary target of t(4;14), we propose that mutated *FGFR3* is a gain-of-function event which leads to worse disease in t(4;14) patients. In the future it will be important to determine if clones with mutated *FGFR3* have a competitive advantage, as evidenced by an increase in the relative proportion of the mutant clones at progression in patients expressing both mutated and unmutated *FGFR3* at baseline. Altogether, these results support the feasibility of FGFR3 inhibitors as potentially invaluable agents targeting a subset of high-risk myeloma patients.

FGFR3 and the t(4;14) Translocation

- Fibroblast growth factor receptor 3 (FGFR3) is a transmembrane cell surface receptor that acts in autocrine and paracrine manners to promote mitogenesis, cell migration, and differentiation.
- Mutations in the immunoglobulin, tansmembrane, and intracellular tyrosine kinase domains have been shown to promote tumorigenesis through constitutive FGFR3 activation.





- In multiple myeloma, a common structural rearrangment occurs between the *IGH* loci on chromosome 14 and the *MMSET/WHSC1/NSD2* locus on chromosome 4, thus giving rise to the t(4;14) translocation.
- In 100% of t(4;14) events, WHSC1 is found to be overexpressed.
- In ~75% of t(4;14) events, FGFR3 expression spikes as a result of its proximity to the IGH 3′ Eu enhancer element.

Results

FGFR3 expression and mutational profiles at baseline for IA11

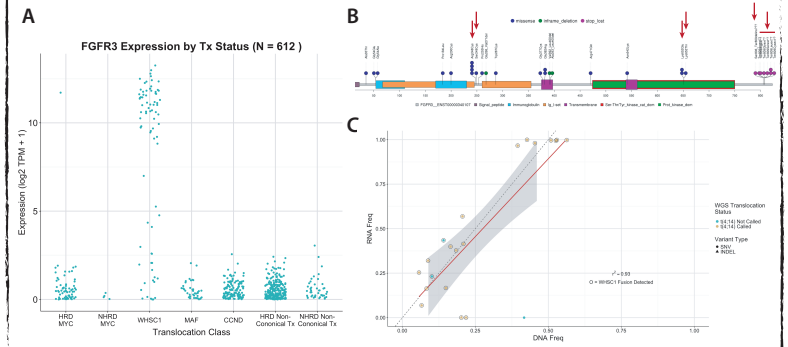


Figure 1. (A) *FGFR3* expression binned by common translocation groups. Spiked expression is observed in t(4;14). (B) Lollipop plot for non-synonymous *FGFR3* mutations. Previously reported activating mutations are depicted with a red arrow. (C) Correlation of DNA and RNA allele frequencies for observed *FGFR3* mutations in patients with RNA-seq and WGS data.

WHSC1 translocations and fusions

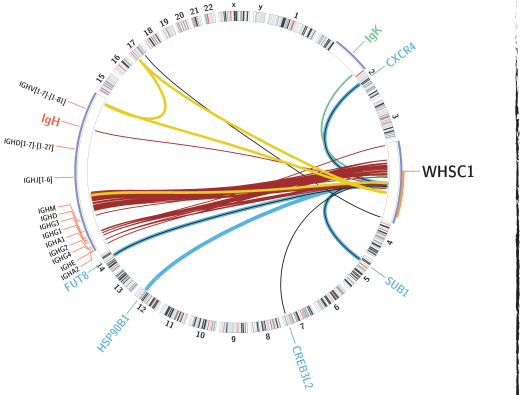


Figure 2. Circos plot for all detected translocations involving *WHSC1*. Canonical t(4;14) IgH translocations are depicted in red. Blue lines indicate novel, non-IgH *WHSC1* translocations while blue lines with black lines inside are called by Delly. The translocation observed involving the Kappa locus is depicted in green. Yellow is a complex translocation to the IGH loci.

t(4;14) characteristics and *FGFR3* mutations

Figure 3. Scatterplot of *FGFR3* and *WHSC1* expression, colored by t(4;14) translocation status. Dashed lines represent RNA expression thresholds for calling t(4;14) translocations if structural data was unavailable. Patients with non-synonymous *FGFR3* mutations are circled in black while those with *WHSC1* mutations are circled in blue. Analysis was constrained to patients with RNA-seq, mutational, and structural data (n = 622).

Survival stratified by t(4;14) status, *FGFR3* expression, and mutation

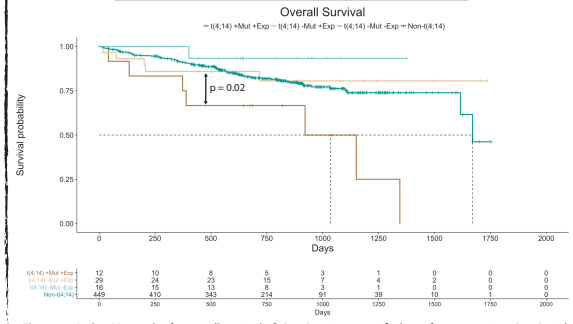


Figure 4. Kaplan-Meyer plot for overall survival of t(4;14) patients stratified into four categories: t(4;14)$^+$ with expressed FGFR3^{mut}, t(4;14)$^+$ with expressed FGFR3^{wt}, t(4;14)$^+$ without *FGFR3* expressed, and t(4;14)$^-$ patients. *FGFR3* mutations appear to drive the poor prognosis associated with t(4;14).

Conclusions

- **WGS detected t(4;14) events in 13% of patients in the baseline cohort of CoMMpass IA11. 72% of t(4;14) tumors express *FGFR3* and 23% of t(4;14) tumors contain non-synonymous, activating *FGFR3* mutations. 10% of t(4;14) patients contain previously reported FGFR3 activating mutations. These results support the continued development of FGFR3 inhibitors as potentially invaluable agents targeting a subset of high-risk myeloma patients.**
- **Novel, non-immunoglobulin translocations were observed between *WHSC1* and *SUB1*, *HSP90B1*, *FUT8*, *CREB3L2*, or *CXCR4*, further demonstrating that *WHSC1* is the target of t(4;14).**
- ***FGFR3* mutations, but not *FGFR3* expression, are an adverse prognostic factor for overall survival in t(4;14) myeloma.**
- **Future sequential analysis of t(4;14)$^+$ tumors will reveal if *FGFR3*^{mut} clones have a competitive growth advantage and/or if spontanious *FGFR3* mutations are aquired over the duration of thereputic intervention.**

PosterCast

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