Discovery and characterization of recurrent, targetable ALK fusions in leiomyosarcoma

Davis et al. (2018)

**SUPPLEMENTARY DATA**

Supplementary Figure S1. ALK copy number data from TCGA-FX-A48G and TCGA-IW-A3M6 LMS visualized using IGV (Broad, MIT)

Supplementary Table S1. Identification of ALK rearrangements using ChimeraScan algorithm.

Supplementary Figure S2. Results from the fusion analysis pipeline run on RNA-seq data for TCGA-FX-A48G identifying KANK2-ALK

Supplementary Figure S3. Results from the fusion analysis pipeline run on RNA-seq data for TCGA-IW-A3M6 identifying ACTG2-ALK

Supplementary Figure S4. Confirmation of KANK2-ALK transgene expression from transformed Ba/F3 cells.

Supplementary Figure S5. Dose-response inhibitor assay with ALK inhibitors with Ba/F3 parental cells in presence of IL-3

Supplementary Figure S6. Body weight of mice treated with vehicle, crizotinib or lorlatinib



**Figure S1.** ALK copy number data from TCGA-FX-A48G and TCGA-IW-A3M6 LMS visualized using IGV. DNA copy number profiles show unbalanced DNA copy numbers at exons 16 and 18 of the ALK gene, respectively. DNA copy number segments were generated by GISTIC algorithm and accessed through cBioPortal (cbioportal.org)

**Supplementary Table S1. Identification of *ALK* rearrangements using ChimeraScan algorithm.**

Genomic coordinates correspond to hg19.



**Supplementary Figure S2. Results from the fusion analysis pipeline run on RNA-seq data for TCGA-FX-A48G identifying KANK2-ALK**

**A) Sequence of the RNA-Seq reads spanning the fusion breakpoint based on Defuse and Chimerascan algorithms**



http://multalin.toulouse.inra.fr

**B) Sequence of KANK2-ALK fusion breakpoint in TCGA-FX-A48G predicted by defuse in whole transcriptome RNA-seq data**

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**C) Complete sequence of KANK2-ALK fusion gene in TCGA-FX-A48G – 4011nt**

**Reference sequences:**

**KANK2**

>NM\_015493.6 Homo sapiens KN motif and ankyrin repeat domains 2 (KANK2), transcript variant 1, mRNA

**ALK**

>NM\_004304.4 Homo sapiens ALK receptor tyrosine kinase (ALK), mRNA

START CODON in frame 2 (position 80, the same as in *KANK2* CDS)

KANK2 – exon 1

KANK2 – exon 2

ALK – exon 16

GTAAGCCTCAGCCGGTGCTGCAGGCAGTCTGACTCGCAGTCCCTCAAGTGACTTCCAAGGAGCATCTGTAGAAAAGAAGATGGCCCAGGTCCTGCACGTGCCTGCTCCCTTCCCAGGGACCCCTGGCCCAGCCTCCCCACCTGCCTTCCCTGCCAAGGACCCCGATCCACCCTACTCCGTGGAGACCCCCTATGGCTACCGCCTGGACCTGGACTTCCTCAAGTACGTGGATGACATCGAGAAGGGCCACACGCTGCGACGCGTGGCAGTGCAGCGCCGCCCCCGCCTGAGCTCGCTGCCCCGTGGCCCTGGCTCCTGGTGGACGTCCACTGAGTCGCTGTGCTCCAATGCCAGTGGGGACAGCCGCCACTCAGCCTATTCCTACTGCGGCCGTGGCTTCTACCCTCAGTATGGTGCTCTGGAGACCCGCGGTGGCTTCAATCCGCGGGTGGAGCGCACGCTGCTGGATGCCCGTCGCCGTCTCGAGGACCAGGCGGCCACACCCACCGGCCTGGGCTCCCTGACCCCCAGTGCGGCCGGCTCGACAGCCTCCCTGGTGGGCGTGGGGTTGCCACCCCCGACACCACGGAGTTCAGGACTGTCCACACCGGTGCCTCCCAGTGCCGGGCACCTGGCCCACGTGCGGGAGCAGATGGCGGGTGCCCTGCGGAAGCTGCGGCAGCTGGAGGAGCAGGTGAAGCTGATCCCTGTGCTCCAGGTGAAGCTCTCGGTGCTCCAGGAGGAAAAGCGGCAGCTCACAGTACAACTTAAGAGCCAGAAGTTCCTGGGCCACCCCACAGCGGGCCGGGGTCGCAGCGAGCTCTGCCTGGACCTCCCCGATCCCCCAGAGGACCCAGTGGCACTGGAGACCCGGAGTGTGGGCACCTGGGTTCGAGAACGGGACTTGGGCATGCCTGATGGGGAGGCTGCCCTCGCCGCCAAGGTCGCTGTGCTGGAGACCCAGCTCAAGAAGGCGCTGCAGGAGCTGCAGGCAGCTCAGGCCCGGCAGGCTGACCCCCAGCCCCAGGCCTGGCCACCGCCGGACAGCCCGGTCCGCGTGGATACAGTCCGGGTGGTAGAAGGGCCACGGGAGGTGGAGGTGGTGGCCAGCACAGCCGCTGGCGCCCCCGCACAGCGGGCCCAGAGCCTGGAGCCTTACGGCACAGGGCTGAGGGCCCTGGCAATGCCTGGTAGGCCTGAGAGCCCACCTGTGTTCCGCAGCCAGGAGGTGGTGGAGACAATGTGCCCAGTGCCCGCTGCAGCTACCAGCAACGTCCATATGGTGAAGAAGATTAGCATCACAGAGCGAAGCTGCGATGGAGCAGCAGGTGGTGGAGGTGGCTGGAATGATAACACTTCCTTGCTCTGGGCCGGAAAATCTTTGCAGGAGGGTGCCACCGGAGGACATTCCTGCCCCCAGGCCATGAAGAAGTGGGGGTGGGAGACAAGAGGGGGTTTCGGAGGGGGTGGAGGGGGGTGCTCCTCAGGTGGAGGAGGCGGAGGATATATAGGCGGCAATGCAGCCTCAAACAATGACCCCGAAATGGATGGGGAAGATGGGGTTTCCTTCATCAGTCCACTGGGCATCCTGTACACCCCAGCTTTAAAAGTGATGGAAGGCCACGGGGAAGTGAATATTAAGCATTATCTAAACTGCAGTCACTGTGAGGTAGACGAATGTCACATGGACCCTGAAAGCCACAAGGTCATCTGCTTCTGTGACCACGGGACGGTGCTGGCTGAGGATGGCGTCTCCTGCATTGTGTCACCCACCCCGGAGCCACACCTGCCACTCTCGCTGATCCTCTCTGTGGTGACCTCTGCCCTCGTGGCCGCCCTGGTCCTGGCTTTCTCCGGCATCATGATTGTGTACCGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAGAGCCCTGAGTACAAGCTGAGCAAGCTCCGCACCTCGACCATCATGACCGACTACAACCCCAACTACTGCTTTGCTGGCAAGACCTCCTCCATCAGTGACCTGAAGGAGGTGCCGCGGAAAAACATCACCCTCATTCGGGGTCTGGGCCATGGCGCCTTTGGGGAGGTGTATGAAGGCCAGGTGTCCGGAATGCCCAACGACCCAAGCCCCCTGCAAGTGGCTGTGAAGACGCTGCCTGAAGTGTGCTCTGAACAGGACGAACTGGATTTCCTCATGGAAGCCCTGATCATCAGCAAATTCAACCACCAGAACATTGTTCGCTGCATTGGGGTGAGCCTGCAATCCCTGCCCCGGTTCATCCTGCTGGAGCTCATGGCGGGGGGAGACCTCAAGTCCTTCCTCCGAGAGACCCGCCCTCGCCCGAGCCAGCCCTCCTCCCTGGCCATGCTGGACCTTCTGCACGTGGCTCGGGACATTGCCTGTGGCTGTCAGTATTTGGAGGAAAACCACTTCATCCACCGAGACATTGCTGCCAGAAACTGCCTCTTGACCTGTCCAGGCCCTGGAAGAGTGGCCAAGATTGGAGACTTCGGGATGGCCCGAGACATCTACAGGGCGAGCTACTATAGAAAGGGAGGCTGTGCCATGCTGCCAGTTAAGTGGATGCCCCCAGAGGCCTTCATGGAAGGAATATTCACTTCTAAAACAGACACATGGTCCTTTGGAGTGCTGCTATGGGAAATCTTTTCTCTTGGATATATGCCATACCCCAGCAAAAGCAACCAGGAAGTTCTGGAGTTTGTCACCAGTGGAGGCCGGATGGACCCACCCAAGAACTGCCCTGGGCCTGTATACCGGATAATGACTCAGTGCTGGCAACATCAGCCTGAAGACAGGCCCAACTTTGCCATCATTTTGGAGAGGATTGAATACTGCACCCAGGACCCGGATGTAATCAACACCGCTTTGCCGATAGAATATGGTCCACTTGTGGAAGAGGAAGAGAAAGTGCCTGTGAGGCCCAAGGACCCTGAGGGGGTTCCTCCTCTCCTGGTCTCTCAACAGGCAAAACGGGAGGAGGAGCGCAGCCCAGCTGCCCCACCACCTCTGCCTACCACCTCCTCTGGCAAGGCTGCAAAGAAACCCACAGCTGCAGAGATCTCTGTTCGAGTCCCTAGAGGGCCGGCCGTGGAAGGGGGACACGTGAATATGGCATTCTCTCAGTCCAACCCTCCTTCGGAGTTGCACAAGGTCCACGGATCCAGAAACAAGCCCACCAGCTTGTGGAACCCAACGTACGGCTCCTGGTTTACAGAGAAACCCACCAAAAAGAATAATCCTATAGCAAAGAAGGAGCCACACGACAGGGGTAACCTGGGGCTGGAGGGAAGCTGTACTGTCCCACCTAACGTTGCAACTGGGAGACTTCCGGGGGCCTCACTGCTCCTAGAGCCCTCTTCGCTGACTGCCAATATGAAGGAGGTACCTCTGTTCAGGCTACGTCACTTCCCTTGTGGGAATGTCAATTACGGCTACCAGCAACAGGGCTTGCCCTTAGAAGCCGCTACTGCCCCTGGAGCTGGTCATTACGAGGATACCATTCTGAAAAGCAAGAATAGCATGAACCAGCCTGGGCCCTGAGCTCGGTCGCACACTCACTTCTCTTCCTTGGGATCCCTAAGACCGTGGAGGAGAGAGAGGCAATGGCTCCTTCACAAACCAGAGACCAAATGTCACGTTTTGTTTTGTGCCAACCTATTTTGAAGTACCACCAAAAAAGCTGTATTTTGAAAATGCTTTAGAAAGGTTTTGAGCATGGGTTCATCCTATTCTTTCGAAAGAAGAAAATATCATAAAAATGAGTGATAAATACAAGGCCCAGATGTGGTTGCATAAGGTTTTTATGCATGTTTGTTGTATACTTCCTTATGCTTCTTTCAAATTGTGTGTGCTCTGCTTCAATGTAGTCAGAATTAGCTGCTTCTATGTTTCATAGTTGGGGTCATAGATGTTTCCTTGCCTTGTTGATGTGGACATGAGCCATTTGAGGGGAGAGGGAACGGAAATAAAGGAGTTATTTGTAATGACTAAAA

**D) KANK2-ALK fusion protein – reading frame preserved, amino acid sequence [from frame 2] – 1159aa**

Start codon

Stop codon

KANK2 portion – 416aa

ALK portion – 743aa

**PTKc domain**

.ASAGAAGSLTRSPSSDFQGASVEKKMAQVLHVPAPFPGTPGPASPPAFPAKDPDPPYSVETPYGYRLDLDFLKYVDDIEKGHTLRRVAVQRRPRLSSLPRGPGSWWTSTESLCSNASGDSRHSAYSYCGRGFYPQYGALETRGGFNPRVERTLLDARRRLEDQAATPTGLGSLTPSAAGSTASLVGVGLPPPTPRSSGLSTPVPPSAGHLAHVREQMAGALRKLRQLEEQVKLIPVLQVKLSVLQEEKRQLTVQLKSQKFLGHPTAGRGRSELCLDLPDPPEDPVALETRSVGTWVRERDLGMPDGEAALAAKVAVLETQLKKALQELQAAQARQADPQPQAWPPPDSPVRVDTVRVVEGPREVEVVASTAAGAPAQRAQSLEPYGTGLRALAMPGRPESPPVFRSQEVVETMCPVPAAATSNVHMVKKISITERSCDGAAGGGGGWNDNTSLLWAGKSLQEGATGGHSCPQAMKKWGWETRGGFGGGGGGCSSGGGGGGYIGGNAASNNDPEMDGEDGVSFISPLGILYTPALKVMEGHGEVNIKHYLNCSHCEVDECHMDPESHKVICFCDHGTVLAEDGVSCIVSPTPEPHLPLSLILSVVTSALVAALVLAFSGIMIVYRRKHQELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDL**KEVPRKNITLIRGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQNIVRCIGVSLQSLPRFILLELMAGGDLKSFLRETRPRPSQPSSLAMLDLLHVARDIACGCQYLEENHFIHRDIAARNCLLTCPGPGRVAKIGDFGMARDIYRASYYRKGGCAMLPVKWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPPKNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEY**CTQDPDVINTALPIEYGPLVEEEEKVPVRPKDPEGVPPLLVSQQAKREEERSPAAPPPLPTTSSGKAAKKPTAAEISVRVPRGPAVEGGHVNMAFSQSNPPSELHKVHGSRNKPTSLWNPTYGSWFTEKPTKKNNPIAKKEPHDRGNLGLEGSCTVPPNVATGRLPGASLLLEPSSLTANMKEVPLFRLRHFPCGNVNYGYQQQGLPLEAATAPGAGHYEDTILKSKNSMNQPGP.

**Supplementary Figure S3. Results from the fusion analysis pipeline run on RNA-seq data for TCGA-IW-A3M6 identifying ACTG2-ALK**

**A) Sequence of the RNA-Seq reads spanning the fusion breakpoint based on Defuse, Chimerascan and FusionCatcher algorithms**

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<http://multalin.toulouse.inra.fr>

**B) Sequence of ACTG2-ALK fusion breakpoint in TCGA-IW-A3M6 predicted by defuse in whole transcriptome RNA-seq data**



**C) Complete sequence of *ACTG2-ALK* fusion gene – 2649nt**

**Reference sequences:**

**ACTG2**

>NM\_001615.3 Homo sapiens actin, gamma 2, smooth muscle, enteric (ACTG2), transcript variant 1, mRNA

**ALK**

>NM\_004304.4 Homo sapiens ALK receptor tyrosine kinase (ALK), mRNA

START CODON in frame 1 (as in the *ACTG2* CDS)

START CODON in frame 2 (preserving ALK sequence in the fusion)

ACTG2 – exon1

ACTG2 – exon 2

ALK – exon 18

GCCTCTGGGGTTTTATATTGCTCTGGTATTCATGCCAAAGACACACCAGCCCTCAGTCACTGGGAGAAGAACCTCTCATACCCTCGGTGCTCCAGTCCCCAGCTCACTCAGCCACACACACCATGTGTGAAGAGGAGACCACCGCGCTCGTGTGTGACAATGGCTCTGGCCTGTGCAAGGCAGGCTTCGCAGGAGATGATGCCCCCCGGGCTGTCTTCCCCTCCATTGTGGGCCGCCCTCGCCACCAGTGATGGAAGGCCACGGGGAAGTGAATATTAAGCATTATCTAAACTGCAGTCACTGTGAGGTAGACGAATGTCACATGGACCCTGAAAGCCACAAGGTCATCTGCTTCTGTGACCACGGGACGGTGCTGGCTGAGGATGGCGTCTCCTGCATTGTGTCACCCACCCCGGAGCCACACCTGCCACTCTCGCTGATCCTCTCTGTGGTGACCTCTGCCCTCGTGGCCGCCCTGGTCCTGGCTTTCTCCGGCATCATGATTGTGTACCGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAGAGCCCTGAGTACAAGCTGAGCAAGCTCCGCACCTCGACCATCATGACCGACTACAACCCCAACTACTGCTTTGCTGGCAAGACCTCCTCCATCAGTGACCTGAAGGAGGTGCCGCGGAAAAACATCACCCTCATTCGGGGTCTGGGCCATGGCGCCTTTGGGGAGGTGTATGAAGGCCAGGTGTCCGGAATGCCCAACGACCCAAGCCCCCTGCAAGTGGCTGTGAAGACGCTGCCTGAAGTGTGCTCTGAACAGGACGAACTGGATTTCCTCATGGAAGCCCTGATCATCAGCAAATTCAACCACCAGAACATTGTTCGCTGCATTGGGGTGAGCCTGCAATCCCTGCCCCGGTTCATCCTGCTGGAGCTCATGGCGGGGGGAGACCTCAAGTCCTTCCTCCGAGAGACCCGCCCTCGCCCGAGCCAGCCCTCCTCCCTGGCCATGCTGGACCTTCTGCACGTGGCTCGGGACATTGCCTGTGGCTGTCAGTATTTGGAGGAAAACCACTTCATCCACCGAGACATTGCTGCCAGAAACTGCCTCTTGACCTGTCCAGGCCCTGGAAGAGTGGCCAAGATTGGAGACTTCGGGATGGCCCGAGACATCTACAGGGCGAGCTACTATAGAAAGGGAGGCTGTGCCATGCTGCCAGTTAAGTGGATGCCCCCAGAGGCCTTCATGGAAGGAATATTCACTTCTAAAACAGACACATGGTCCTTTGGAGTGCTGCTATGGGAAATCTTTTCTCTTGGATATATGCCATACCCCAGCAAAAGCAACCAGGAAGTTCTGGAGTTTGTCACCAGTGGAGGCCGGATGGACCCACCCAAGAACTGCCCTGGGCCTGTATACCGGATAATGACTCAGTGCTGGCAACATCAGCCTGAAGACAGGCCCAACTTTGCCATCATTTTGGAGAGGATTGAATACTGCACCCAGGACCCGGATGTAATCAACACCGCTTTGCCGATAGAATATGGTCCACTTGTGGAAGAGGAAGAGAAAGTGCCTGTGAGGCCCAAGGACCCTGAGGGGGTTCCTCCTCTCCTGGTCTCTCAACAGGCAAAACGGGAGGAGGAGCGCAGCCCAGCTGCCCCACCACCTCTGCCTACCACCTCCTCTGGCAAGGCTGCAAAGAAACCCACAGCTGCAGAGATCTCTGTTCGAGTCCCTAGAGGGCCGGCCGTGGAAGGGGGACACGTGAATATGGCATTCTCTCAGTCCAACCCTCCTTCGGAGTTGCACAAGGTCCACGGATCCAGAAACAAGCCCACCAGCTTGTGGAACCCAACGTACGGCTCCTGGTTTACAGAGAAACCCACCAAAAAGAATAATCCTATAGCAAAGAAGGAGCCACACGACAGGGGTAACCTGGGGCTGGAGGGAAGCTGTACTGTCCCACCTAACGTTGCAACTGGGAGACTTCCGGGGGCCTCACTGCTCCTAGAGCCCTCTTCGCTGACTGCCAATATGAAGGAGGTACCTCTGTTCAGGCTACGTCACTTCCCTTGTGGGAATGTCAATTACGGCTACCAGCAACAGGGCTTGCCCTTAGAAGCCGCTACTGCCCCTGGAGCTGGTCATTACGAGGATACCATTCTGAAAAGCAAGAATAGCATGAACCAGCCTGGGCCCTGAGCTCGGTCGCACACTCACTTCTCTTCCTTGGGATCCCTAAGACCGTGGAGGAGAGAGAGGCAATGGCTCCTTCACAAACCAGAGACCAAATGTCACGTTTTGTTTTGTGCCAACCTATTTTGAAGTACCACCAAAAAAGCTGTATTTTGAAAATGCTTTAGAAAGGTTTTGAGCATGGGTTCATCCTATTCTTTCGAAAGAAGAAAATATCATAAAAATGAGTGATAAATACAAGGCCCAGATGTGGTTGCATAAGGTTTTTATGCATGTTTGTTGTATACTTCCTTATGCTTCTTTCAAATTGTGTGTGCTCTGCTTCAATGTAGTCAGAATTAGCTGCTTCTATGTTTCATAGTTGGGGTCATAGATGTTTCCTTGCCTTGTTGATGTGGACATGAGCCATTTGAGGGGAGAGGGAACGGAAATAAAGGAGTTATTTGTAATGACTAAAA

**D) Product of ACTG2-ALK fusion – amino acid sequence from frame 2, ALK portion translated – 648aa**

Start codon

Stop codon

Exons 18-29 of ALK preserved

**PTKc domain**

PLGFYIALVFMPKTHQPSVTGRRTSHTLGAPVPSSLSHTHHV.RGDHRARV.QWLWPVQGRLRRR.CPPGCLPLHCGPPSPPVMEGHGEVNIKHYLNCSHCEVDECHMDPESHKVICFCDHGTVLAEDGVSCIVSPTPEPHLPLSLILSVVTSALVAALVLAFSGIMIVYRRKHQELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDL**KEVPRKNITLIRGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQNIVRCIGVSLQSLPRFILLELMAGGDLKSFLRETRPRPSQPSSLAMLDLLHVARDIACGCQYLEENHFIHRDIAARNCLLTCPGPGRVAKIGDFGMARDIYRASYYRKGGCAMLPVKWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPPKNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEY**CTQDPDVINTALPIEYGPLVEEEEKVPVRPKDPEGVPPLLVSQQAKREEERSPAAPPPLPTTSSGKAAKKPTAAEISVRVPRGPAVEGGHVNMAFSQSNPPSELHKVHGSRNKPTSLWNPTYGSWFTEKPTKKNNPIAKKEPHDRGNLGLEGSCTVPPNVATGRLPGASLLLEPSSLTANMKEVPLFRLRHFPCGNVNYGYQQQGLPLEAATAPGAGHYEDTILKSKNSMNQPGP.ARSHTHFSSLGSLRPWRRERQWLLHKPETKCHVLFCANLF.STTKKAVF.KCFRKVLSMGSSYSFERRKYHKNE..IQGPDVVA.GFYACLLYTSLCFFQIVCALLQCSQN.LLLCFIVGVIDVSLPC.CGHEPFEGRGNGNKGVICND.

**Supplementary Figure S4.** Confirmation of KANK2-ALK expression in transformed Ba/F3 KANK2-ALK cell line



Primer sequences:

KANK2\_Fwd (exon 2): 5’ GAGGTGGTGGAGACAATGTG 3’

ALK\_Rev (exon 21): 5’ GACACCTGGCCTTCATACAC 3’

**Supplementary Figure S5.** Dose-response inhibitor assay with ALK inhibitors with Ba/F3 parental cells in presence of IL-3, demonstrating no significant off-target cytotoxicity.

**+Interleukin-3**

**Supplementary Figure S6.** Body weight of mice treated with vehicle, crizotinib or lorlatinib from Day 0 of treatment (Tx) to time of euthanasia or censorship of data

