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Revisiting the Correlation of *c-kit* Mutation Status and Treatment Decisions in Canine Mast Cell Tumors

Department of Pathobiology
Institute of Immunology

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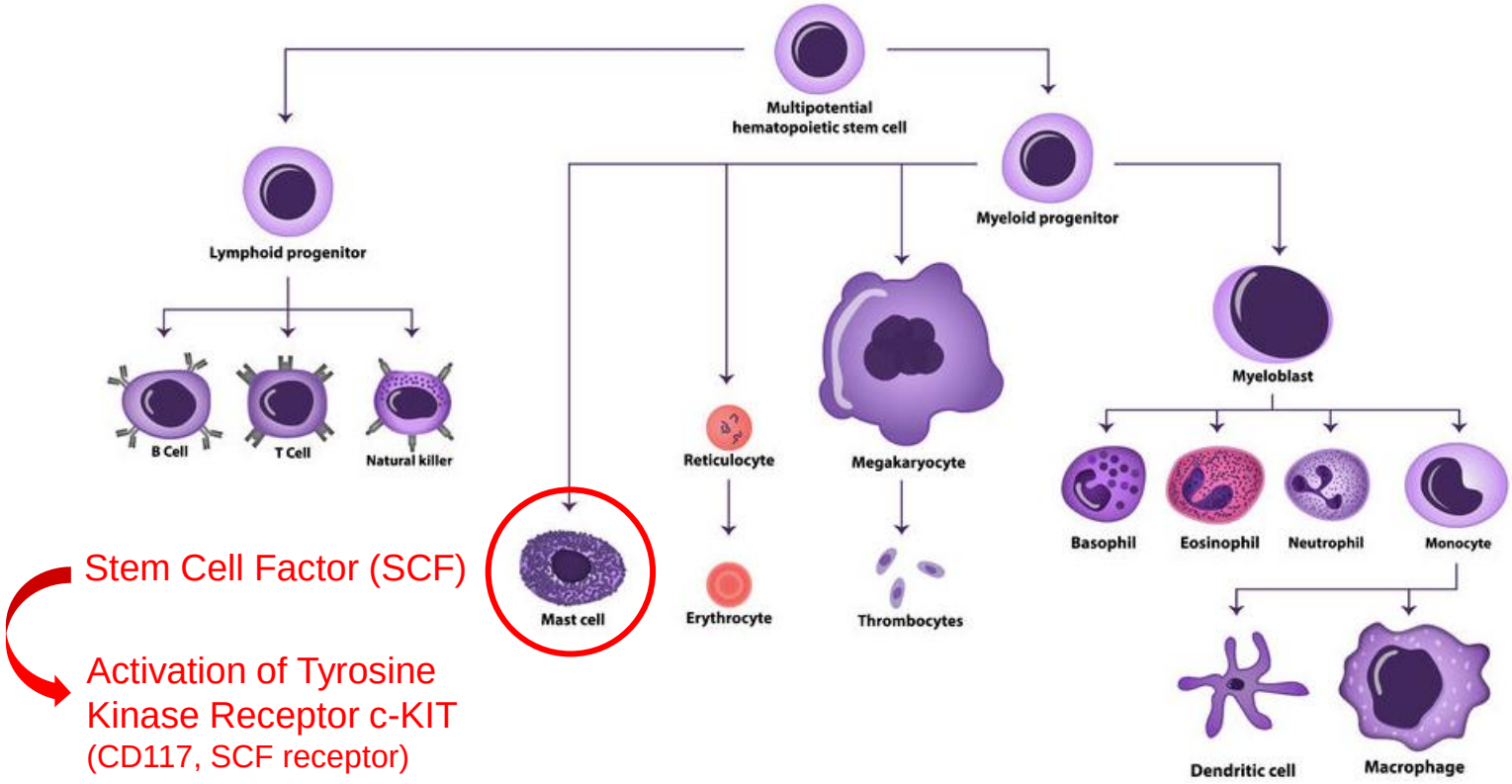
Focused Scientific Session: Diagnostic Pathology
November 14, 2022 | 8.00 – 9.45 a.m.

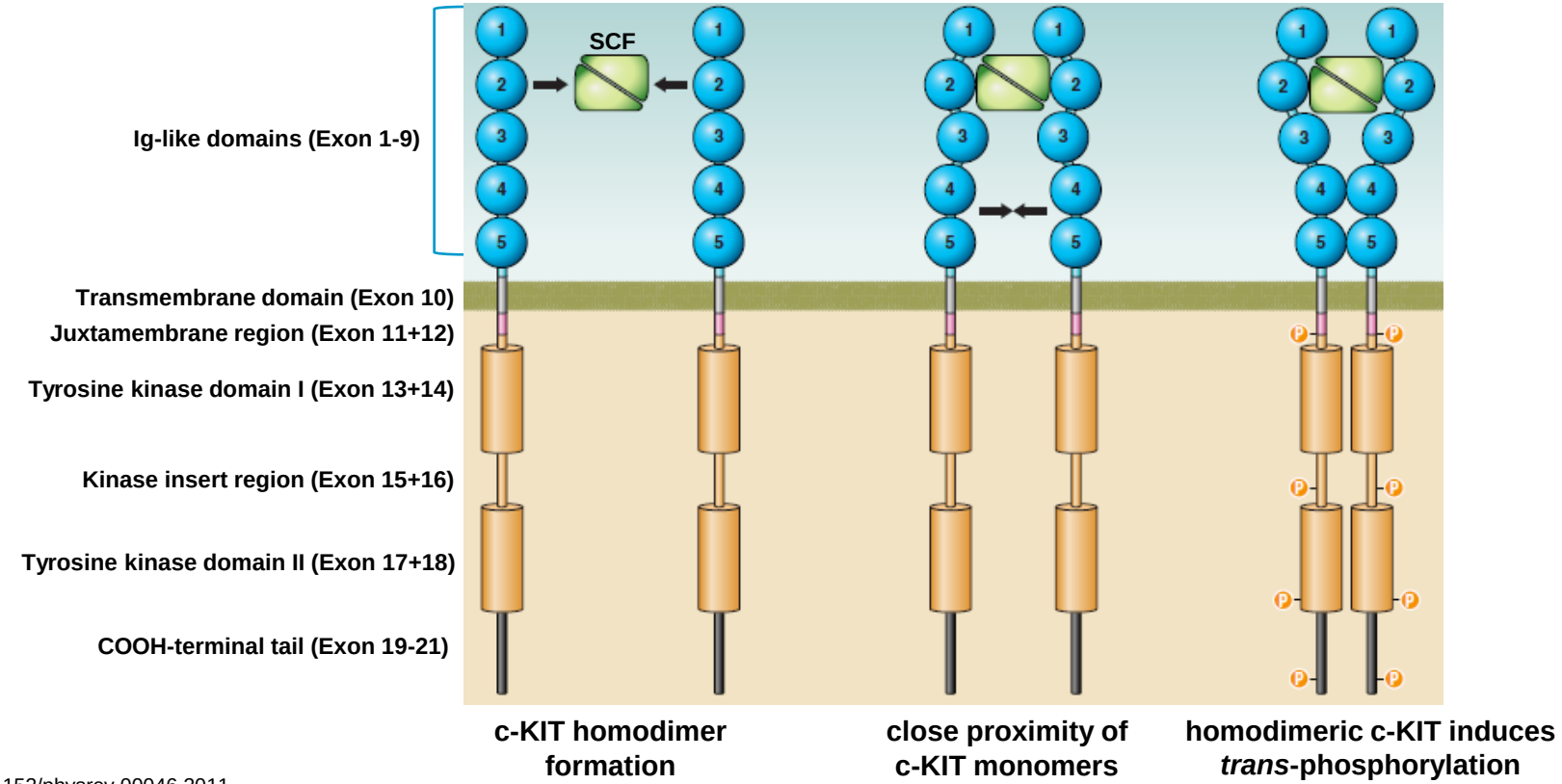
Relevant Financial Relationship Disclosure Statement

In relation to this presentation, I do not have any relationships with companies to report.

Mast Cells express Tyrosine Kinase Receptor c-KIT

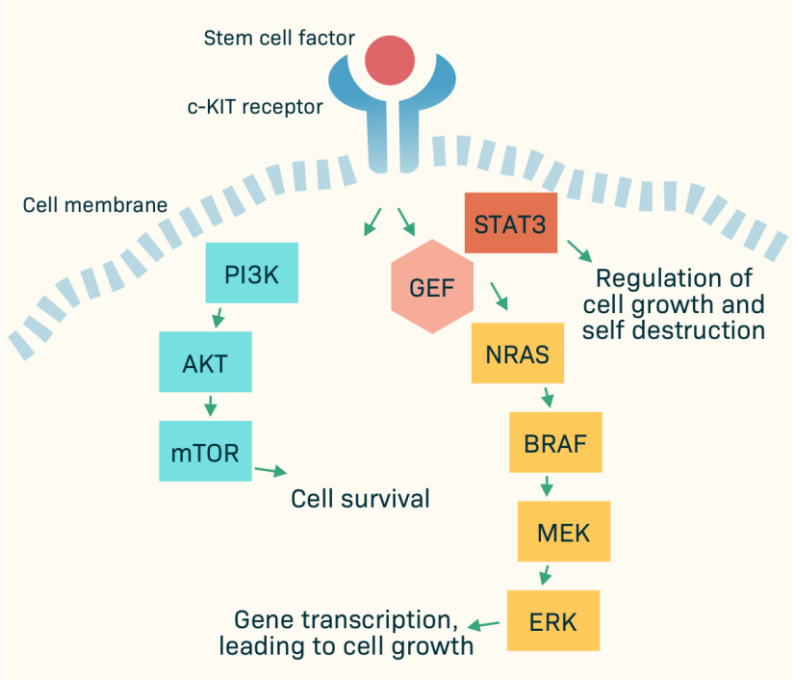


Stem cell factor (SCF) induced c-KIT activation

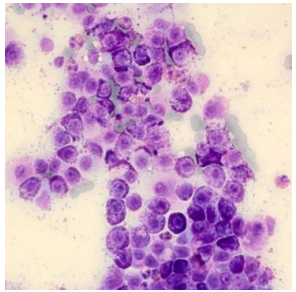


DOI:10.1152/physrev.00046.2011

Stem cell factor (SCF) receptor encoded by *c-kit*



- Mutations in the proto-oncogene *c-kit*, encodes for the SCF receptor on the surface of mast cells → induction of constitutive receptor activation

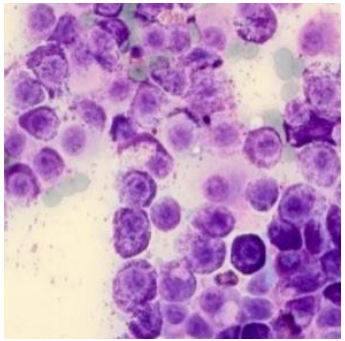


- Known mutations
  - Internal tandem duplication (ITD)
  - Single nucleotid polymorphism (SNP)
  - Insertions, Deletions
- Literature: 50% of MCTs in the dog show an ITD in Exon 8 or 11

Gleevec (imatinib)

Mast cell tumors in dog

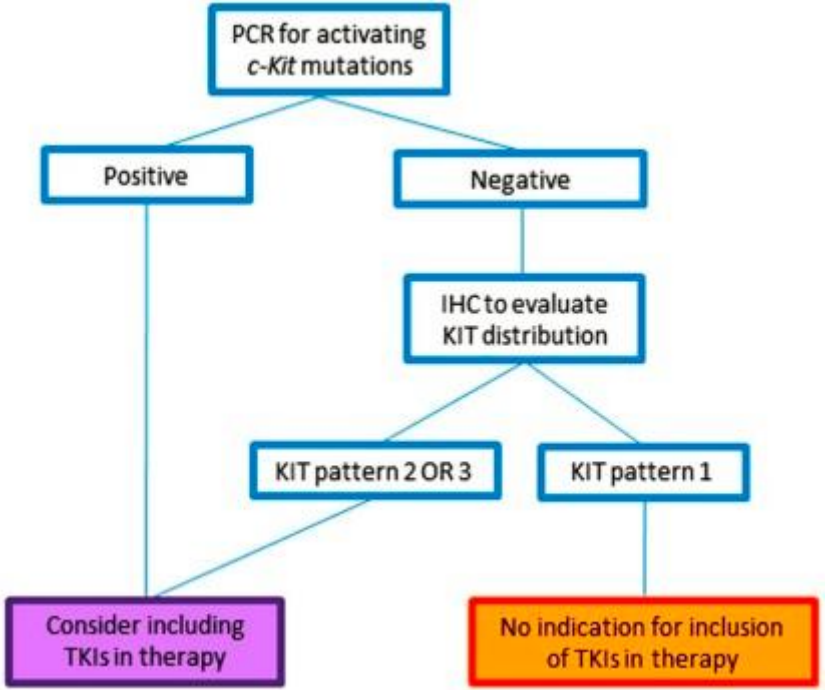
- very common – incidence 16-21% of all skin neoplasias
- breed disposition



- skin solitary mass, multiple possible – "wax and wain"
- diagnosis in 92-96% of cases using cytology
  no classification, no prognosis – description of malignant morphology
- classification: histopathology – Patnaik (I, II, II), Kiupel (low grade, high grade)
- therapy – surgery
  recidive vs systemic disease –
  chemotherapy, Tyrosine Kinase Inhibitors (TKIs)

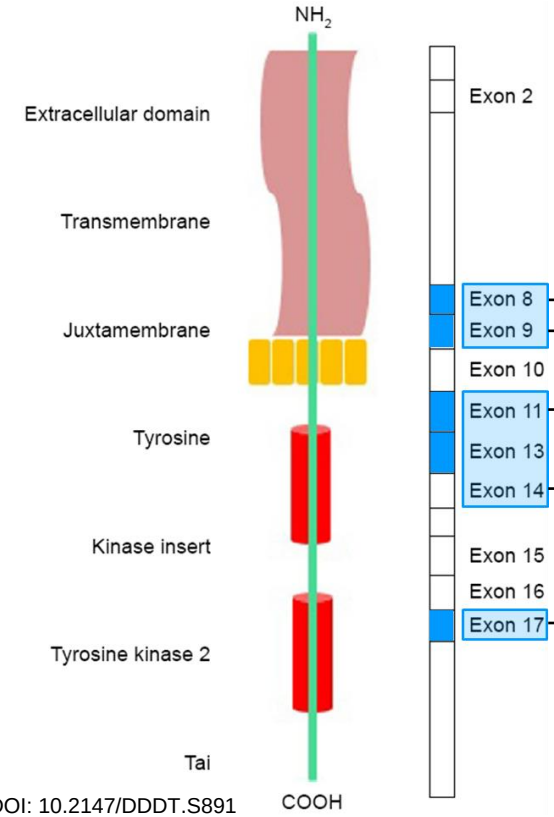
Do Tyrosine Kinase Inhibitors (TKIs) make sense?

- TKIs expand the treatment possibilities in MCT
- TKIs selectively bind to SCF receptor and inhibit its overactivation
- Is it useful to test the *c-kit* mutation status prior to MCT treatment with TKIs?
  - Yes – increased effect if activating mutation is present
  - Side effects may occur, as well as spontaneous mutations or tumor resistance



DOI:10.1016/j.tvjl.2016.06.003

c-kit mutation screening



TKIs

Q430R, ITD, deletions → constitutive activation of c-KIT protein

S471I, N508I → constitutive activation of c-KIT protein
A510V → TKI resistance

ITD → constitutive activation of c-KIT protein

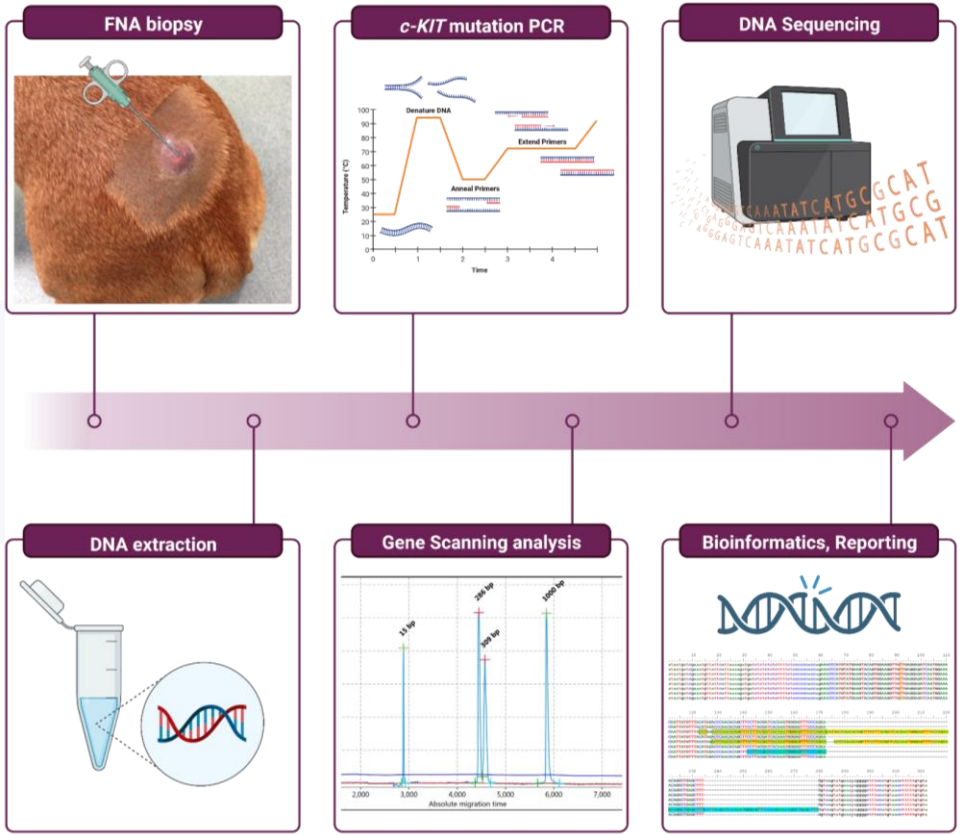
T669I → TKI resistance

A813P → pathogenic
D815H → TKI resistance

DOI: 10.2147/DDDT.S891

TKIs, Tyrosine Kinase Inhibitors

c-kit mutation analysis workflow



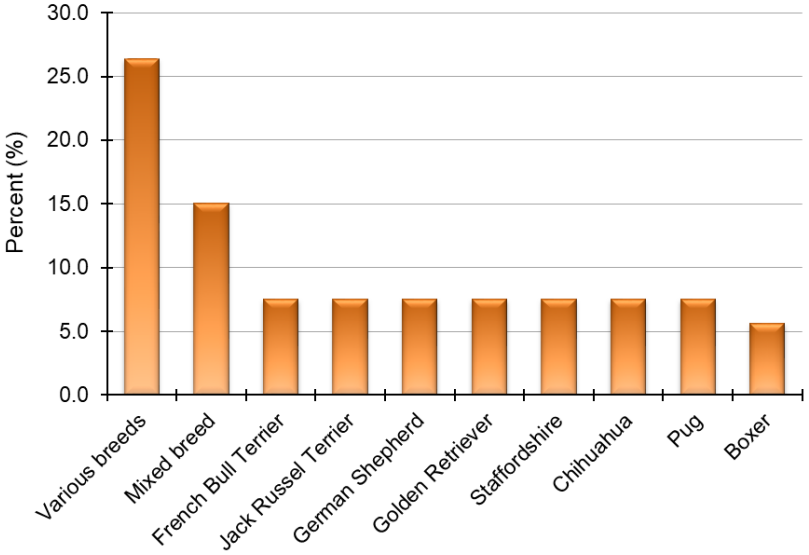
- single cell suspension (cooled or RT) as for FCM – if viable also other possibilities
- stained/unstained cytology slides – history, localization, morphology, DNA quality/amount
- histopathology samples (50µm) – history, localization, cytology report etc.

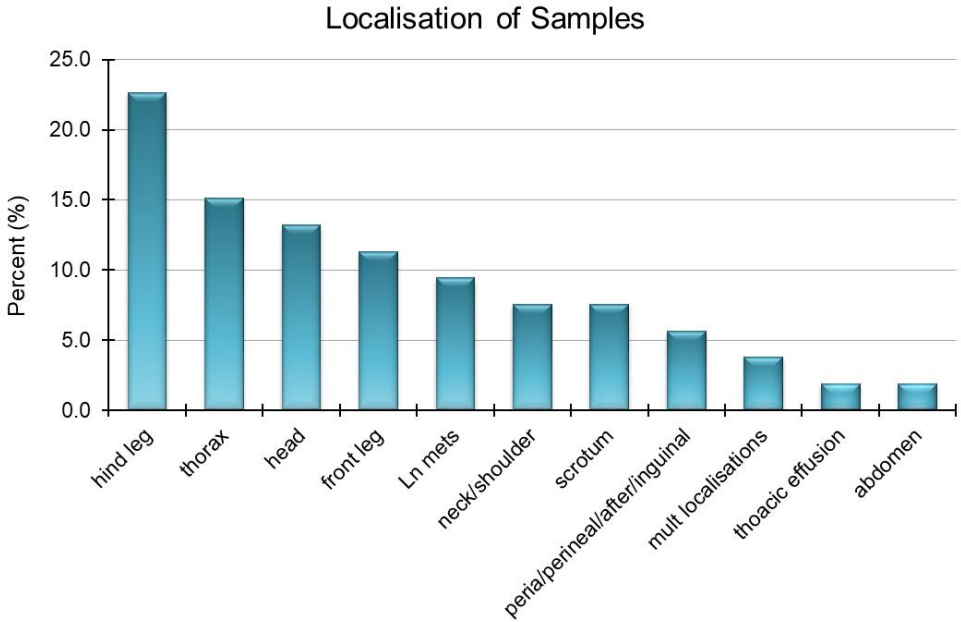
- Analysis is performed every 2nd week
- Workflow takes 4 business days
- Total turn-around-time: 14 days

FCM, Flow cytometry; RT, room temperature

Data collected (1)

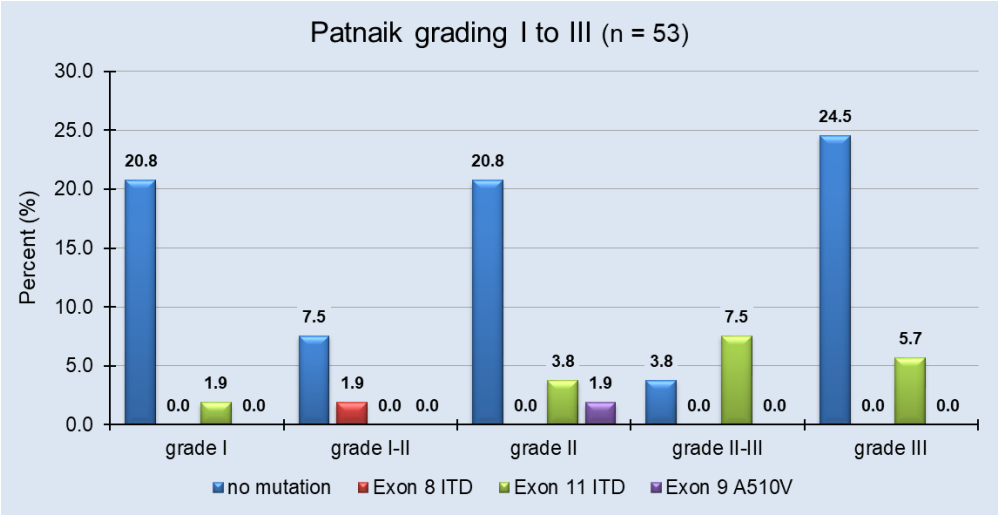
53 MCT samples - *c-kit* exons 8, 9, 11, 13, 14 and 17 analyzed

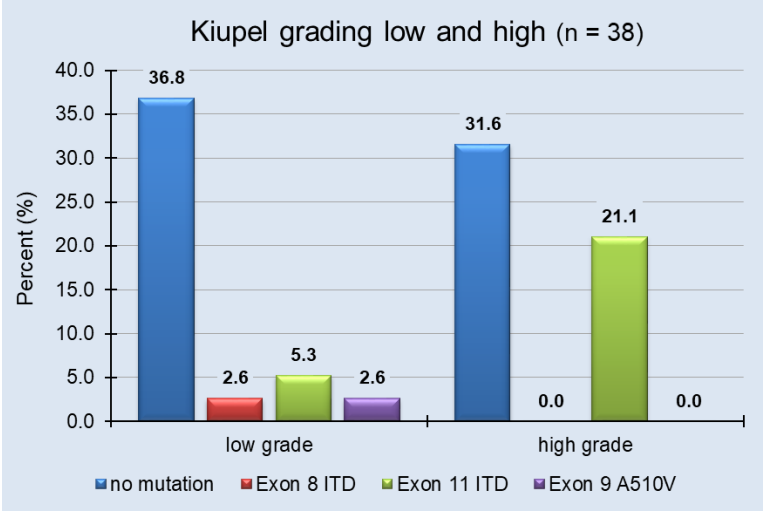




Data collected (2)

53 MCT samples - *c-kit* exons 8, 9, 11, 13, 14 and 17 analyzed
– 38 with Patnaik and Kiupel grading

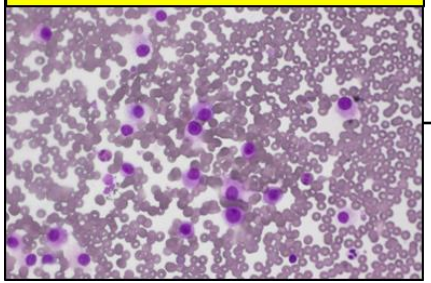


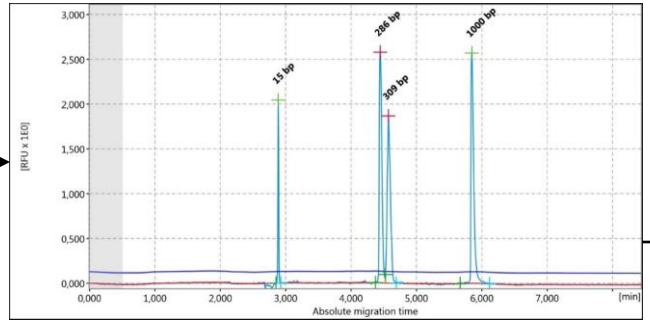


Only 20.7% patients showed an ITD in Exon 8 or 11

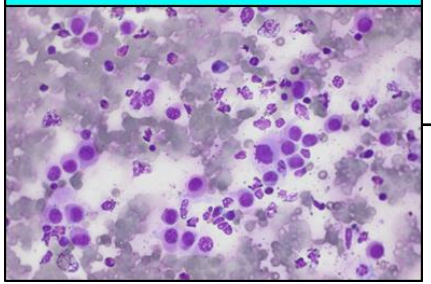
Two examples of activating mutation (ITD) in *c-kit* Exon 11 (1)

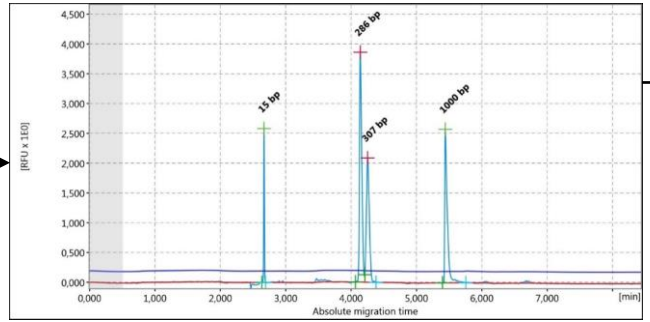
61: inguinal ln. Mutation: ITD exon 11





43: mandibular ln. Mutation: ITD exon 11

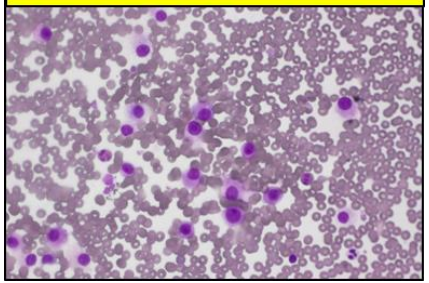




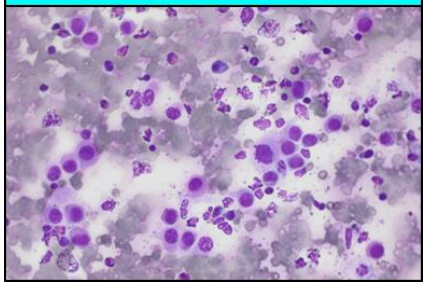
ITD, Internal tandem duplication

Two examples of activating mutation (ITD) in *c-kit* Exon 11 (2)

61: inguinal ln. Mutation: ITD exon 11



43: mandibular ln. Mutation: ITD exon 11



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                                10        20        30        40        50        60        70        80        90        100       110
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Dog Wild type                   ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA
C2 Wild type                    ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA
C2 ITD exon 11                  ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA
61 Wild type                    ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA
61 ITD exon 11                  ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA
43 Wild type                    ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA
43 ITD exon 11                  ataatgatcgaaatgttattcattaaaagatgatctrtctctcttttctcccccccaccagAAACCCATGTATGAAGTACAGTGGAAGGTTGTTGAGGAGATCAATGGAAA

                                120       130       140       150       160       170       180       190       200       210       220
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Dog Wild type                   CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA----------------------------------------------------
C2 Wild type                    CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA----------------------------------------------------
C2 ITD exon 11                  CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAACATACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA
61 Wild type                    CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA----------------------------------------------------
61 ITD exon 11                  CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA---ACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA
43 Wild type                    CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA----------------------------------------------------
43 ITD exon 11                  CAATTATGTTTACATAGACCCAACACAGCTTCCTTACGATCACAAATGGGAGTTTCCCAGAA----------------------------------------------------

                                230       240       250       260       270       280       290       300       310       320
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|..
Dog Wild type                   ACAGGCTGAGCTTT----------------------------------------------Ggtcagtatgaaayaggggctttccatgtaacctttttgtgta
C2 Wild type                    ACAGGCTGAGCTTT----------------------------------------------Ggtcagtatgaaayaggggctttccatgtaacctttttgtgta
C2 ITD exon 11                  ACAGGCTGAGCTTT----------------------------------------------Ggtcagtatgaaayaggggctttccatgtaacctttttgtgta
61 Wild type                    ACAGGCTGAGCTTT----------------------------------------------Ggtcagtatgaaayaggggctttccatgtaacctttttgtgta
61 ITD exon 11                  ACAGGCTGAGCTTT----------------------------------------------Ggtcagtatgaaayaggggctttccatgtaacctttttgtgta
43 Wild type                    ACAGGCTGAGCTTT----------------------------------------------Ggtcagtatgaaayaggggctttccatgtaacctttttgtgta
43 ITD exon 11                  ACAGGCTGAGCTTTCCTTACGATCACAAATGGGAGTTTCCCAGAAACAGGCTGAGCTTTGgtcagtatgaaayaggggctttccatgtaacctttttgtgta
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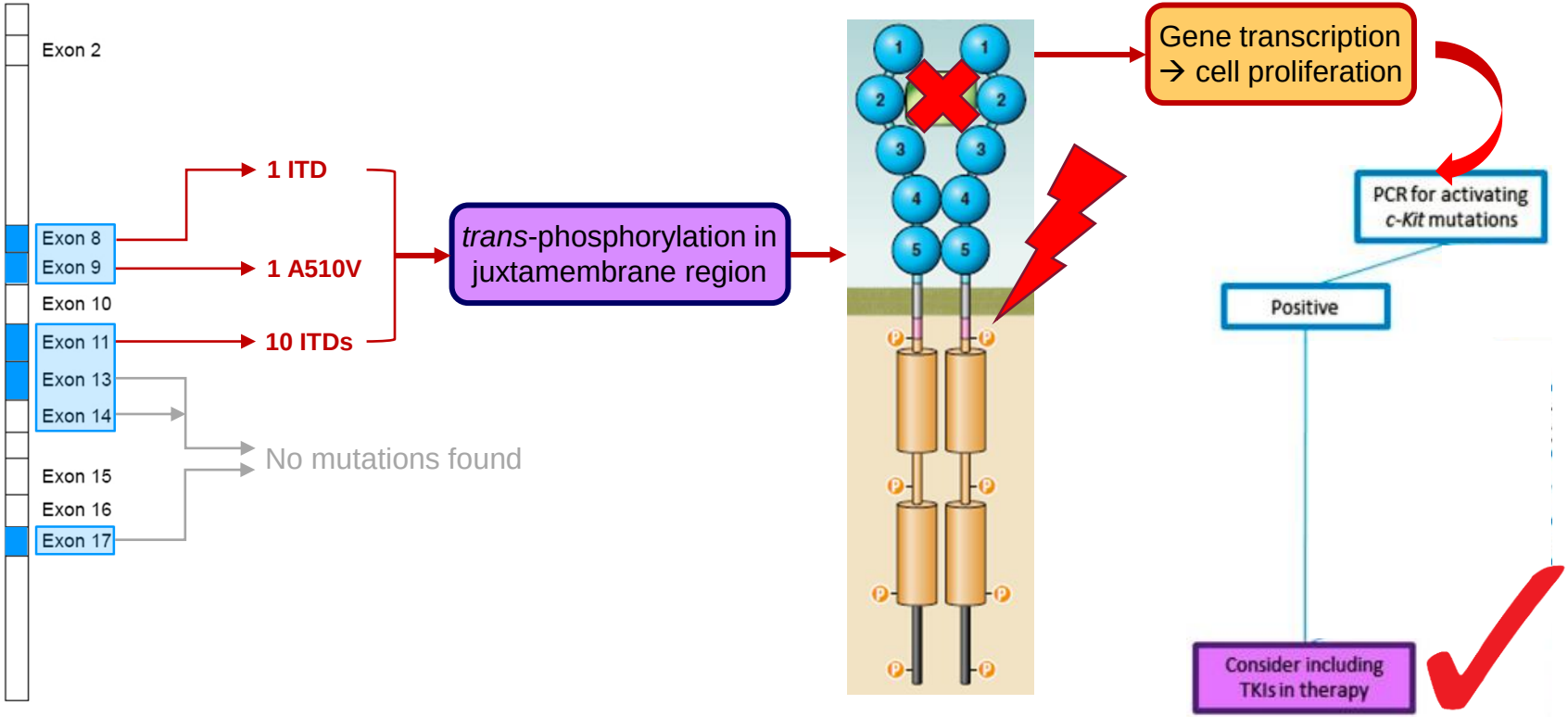
Histopathology: high-grade (Kiupel) | grade III (Patnaik)

ITD, Internal tandem duplication

Outcome *c-kit* mutation screening



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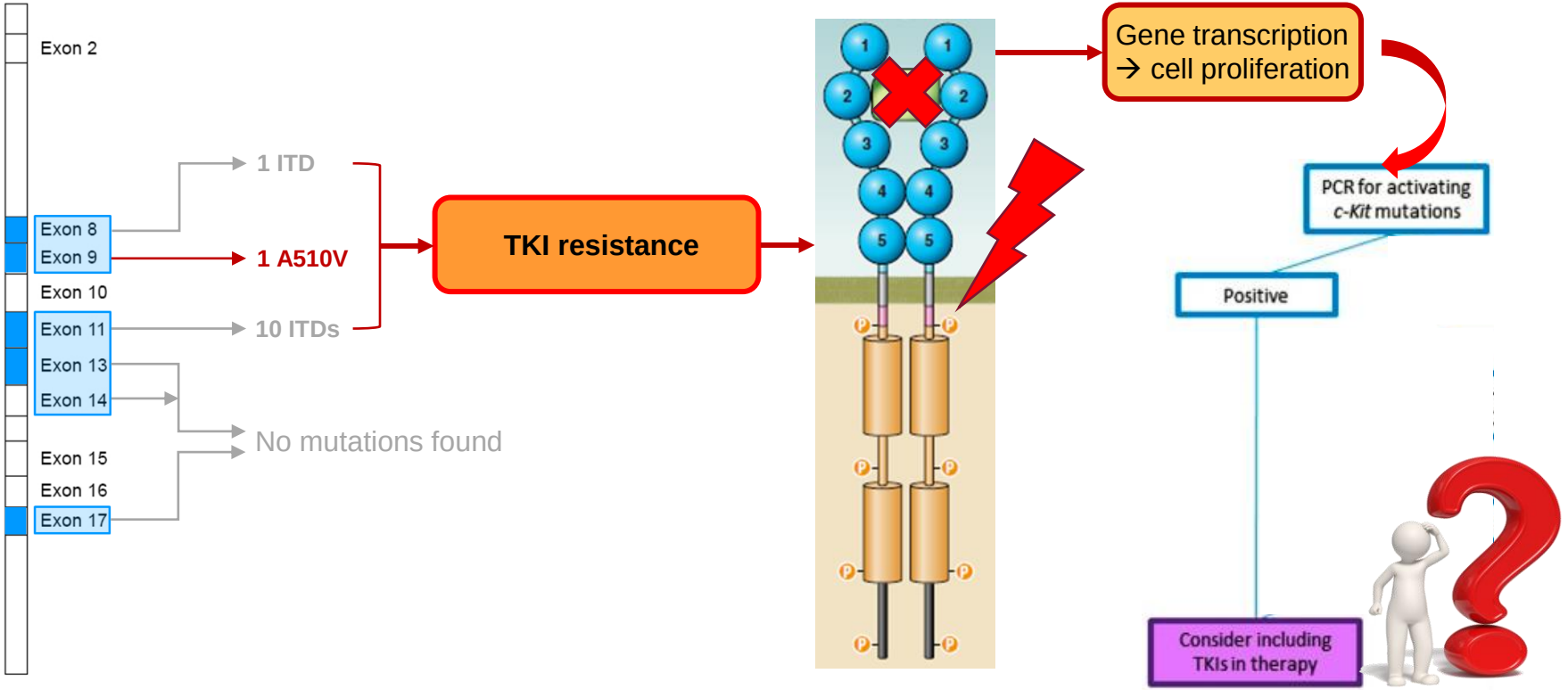

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Acknowledgements

My colleagues at the Clinical Pathology, Institute of Immunology and Institute of Pathology in Vienna
Our students Amina, Viktoria and Giovanni

Barbara C. Rütgen

Thank you for your attention!

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