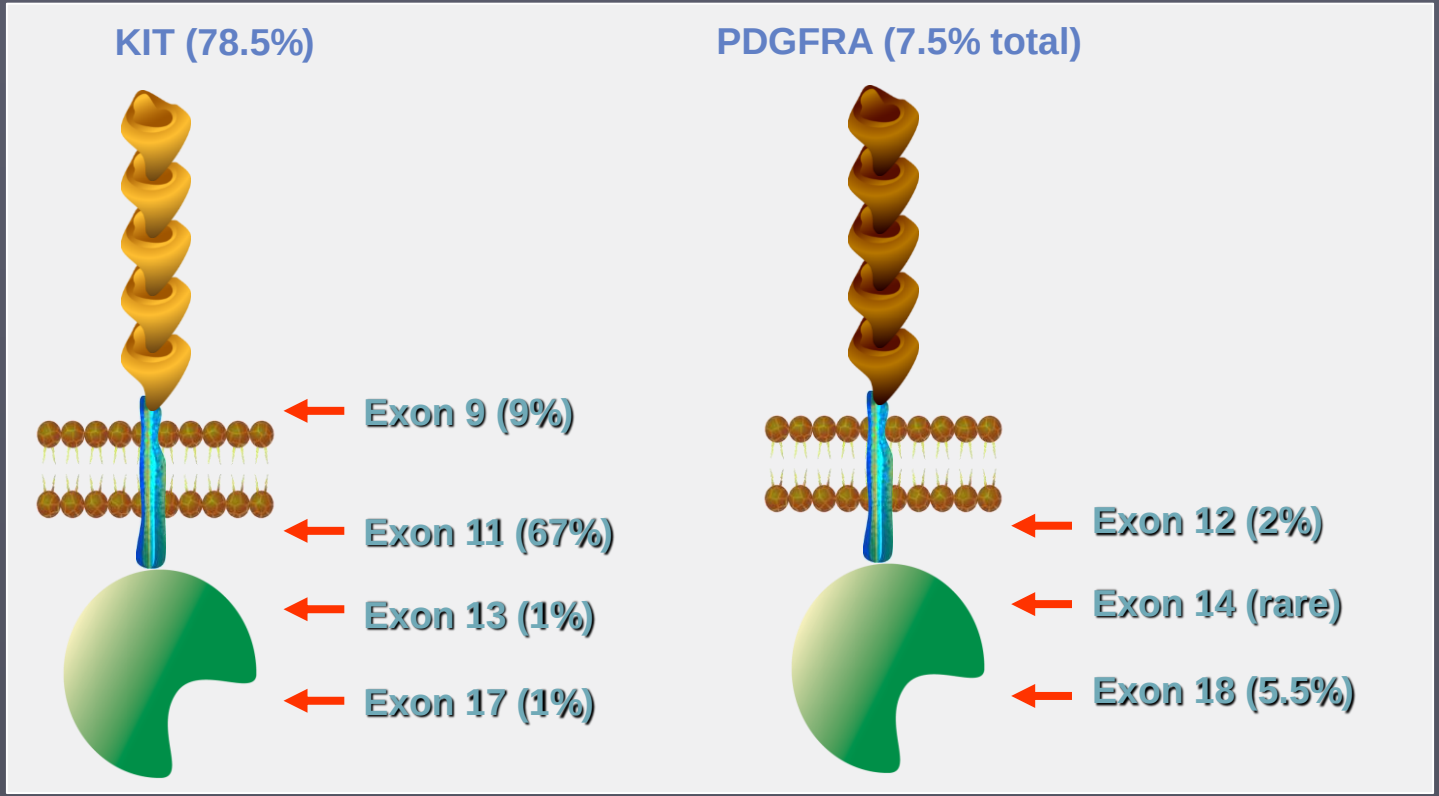
Jonathan Fletcher Lab
Brigham and Women's Hospital, Harvard Medical School

Discoveries and Successes in GIST Basic Research

Dana-Farber/Harvard Cancer Center

KIT/PDGFRA activation (or NF1 inactivation) Kinase proliferation pathways



SDH-deficient GISTs

- Most SDH-deficient GISTs have mutations in one of the 4 SDH genes
- Other SDH-deficient GISTs have EPIGENETIC alterations that inactivate the SDHC gene (e.g., in Carney triad GISTs)
- Unique biology among GISTs = genome hypermethylation

GIST Immunotherapies

- Immune checkpoint drugs = little/no clinical activity as single-agents.

- Lab studies: immunotherapies might have better success in combination with KIT/PDGFRA inhibition (imatinib)

GIST Genetic (Genomic) Progression

- How a GIST progresses from benign tumor to cancer, after the initiating KIT, PDGFRA or NF1 mutation

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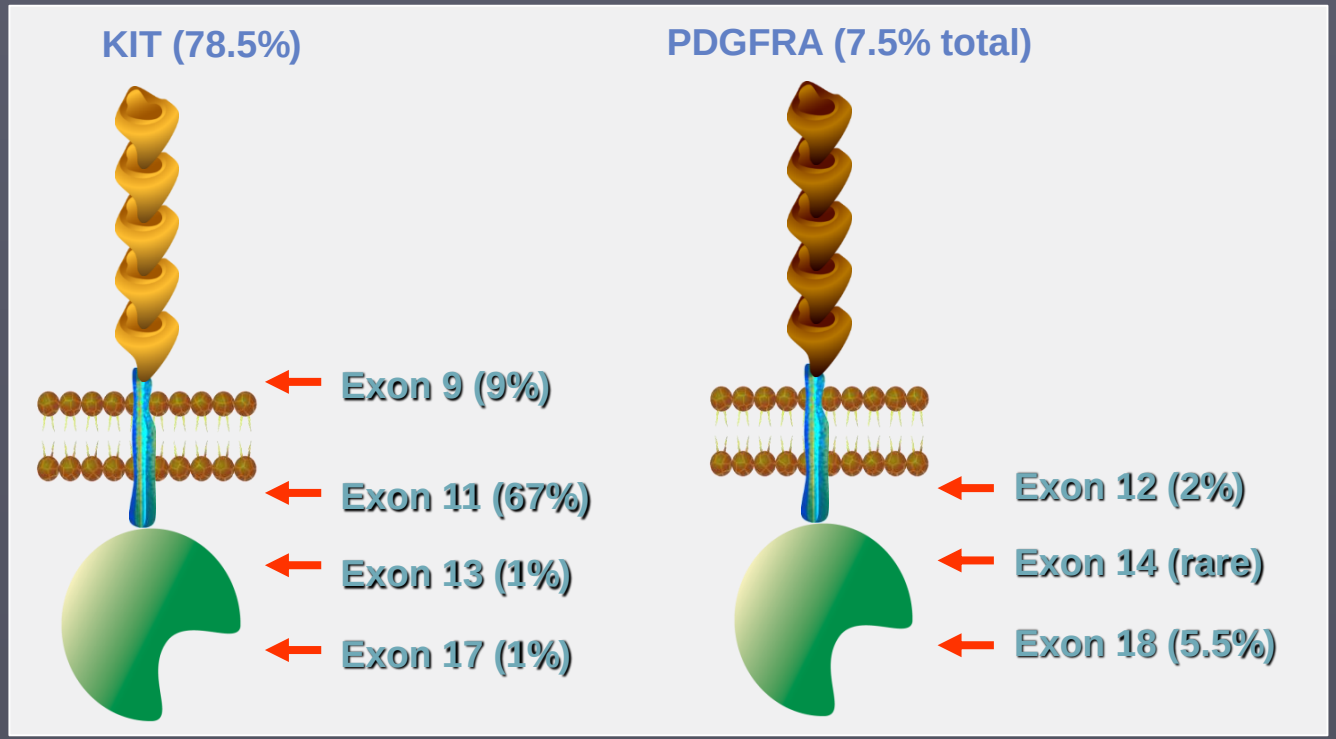
MicroGIST to Metastatic Imatinib-resistant GIST (progression in 8 genomic steps)

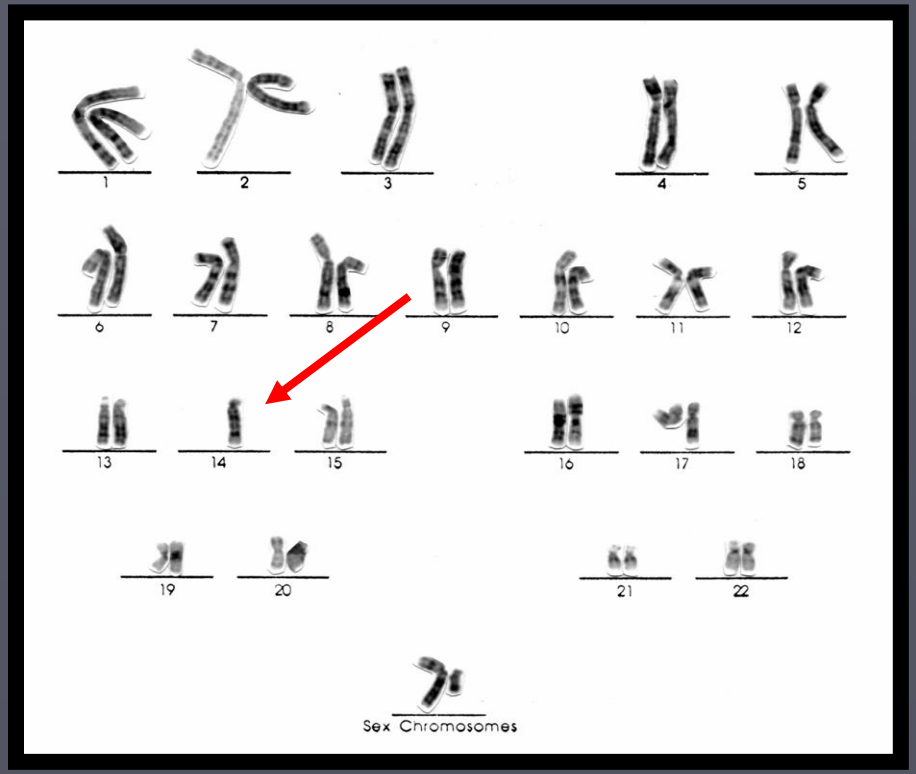
Dana-Farber/Harvard Cancer Center

Step 1: KIT/PDGFRA activation or NF1 inactivation



GIST: building on 14q deletion

A



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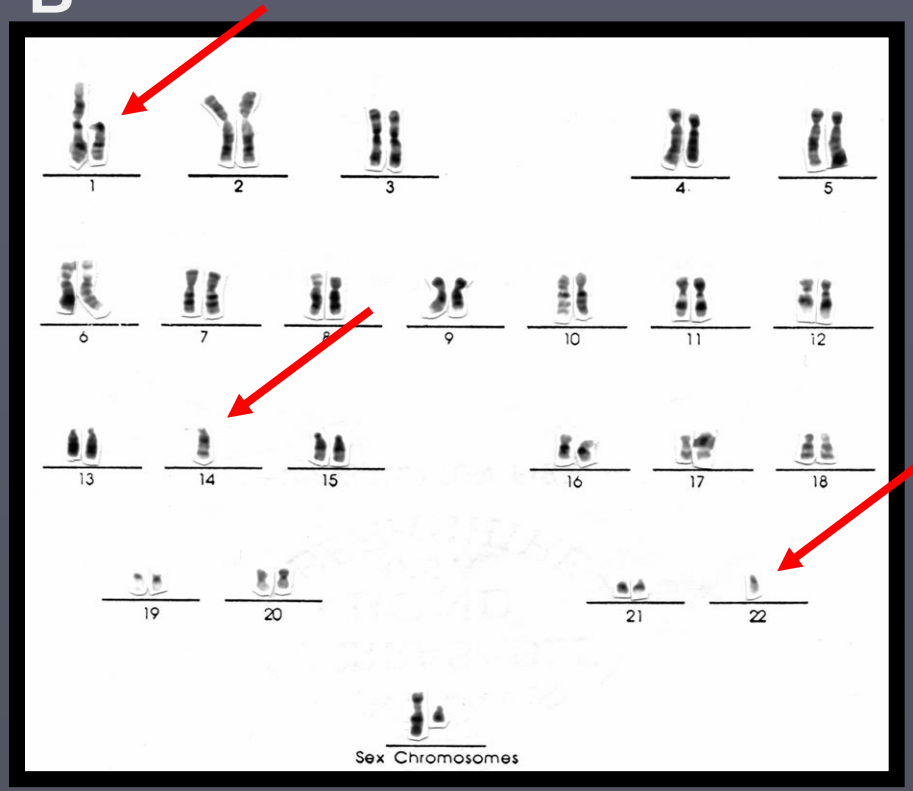
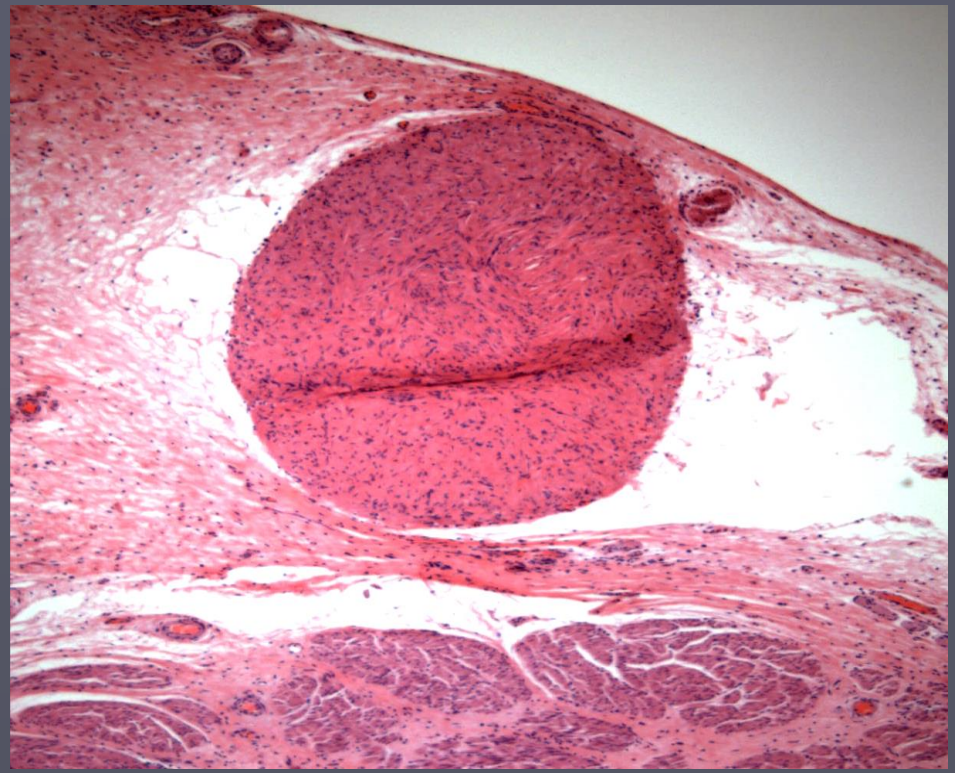


KIT → 14q > 22q > 1p > cell cycle > 15q > dystrophin

KIT/PDGFRA-mutant microGIST (<1cm)

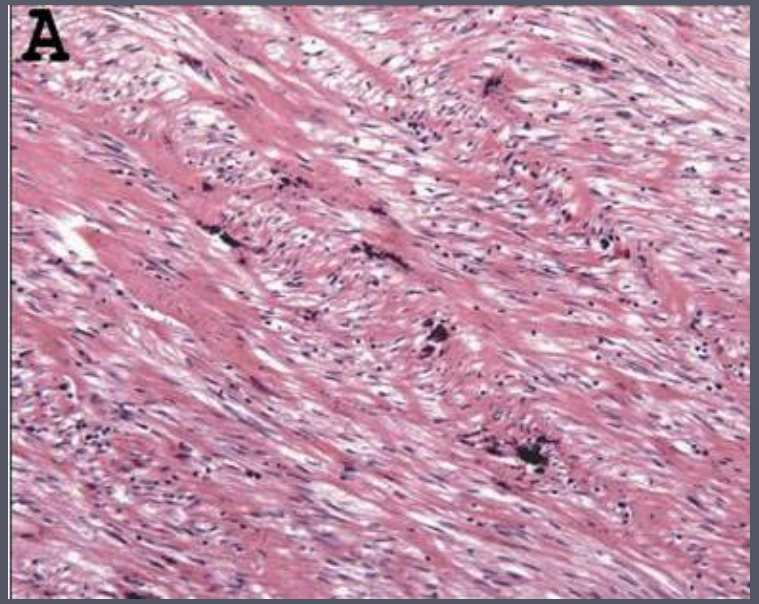
Found in 30% of the general population



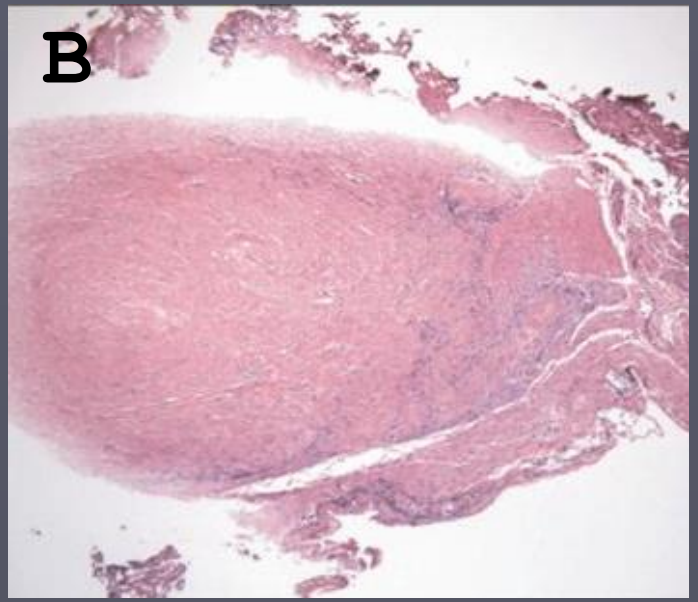
EG junction, spindle cell type

Dr. Cher-Wei Liang

Despite having KIT & PDGFRA mutations, MicroGISTs struggle to grow



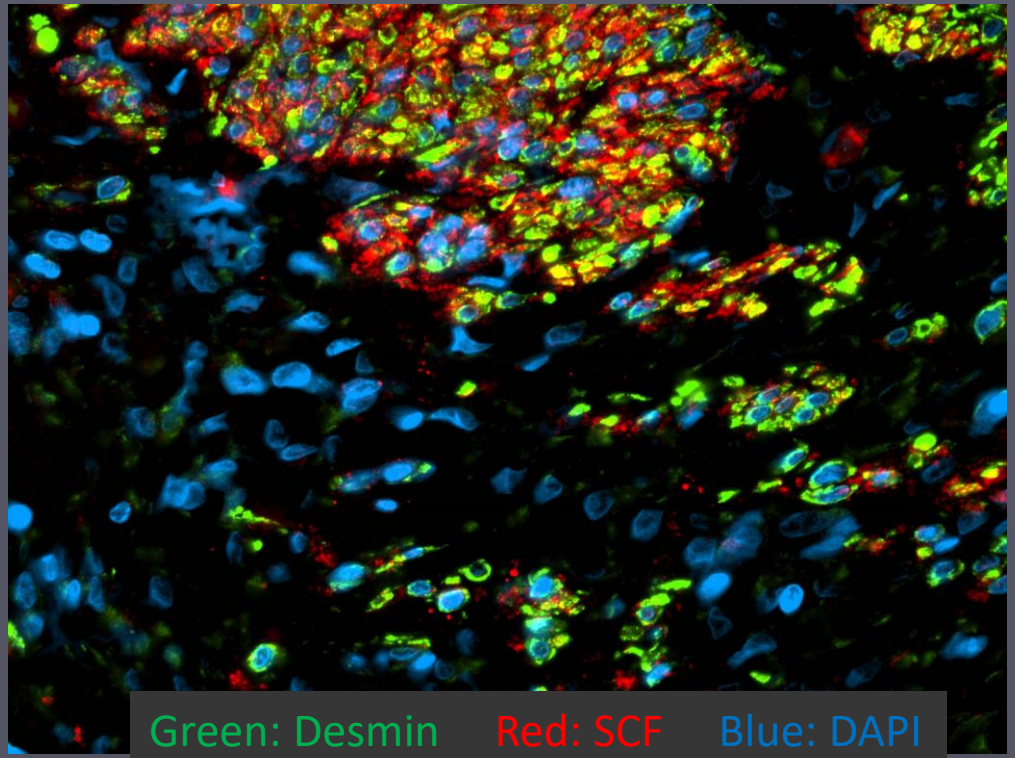
Calcification



Hyalinization

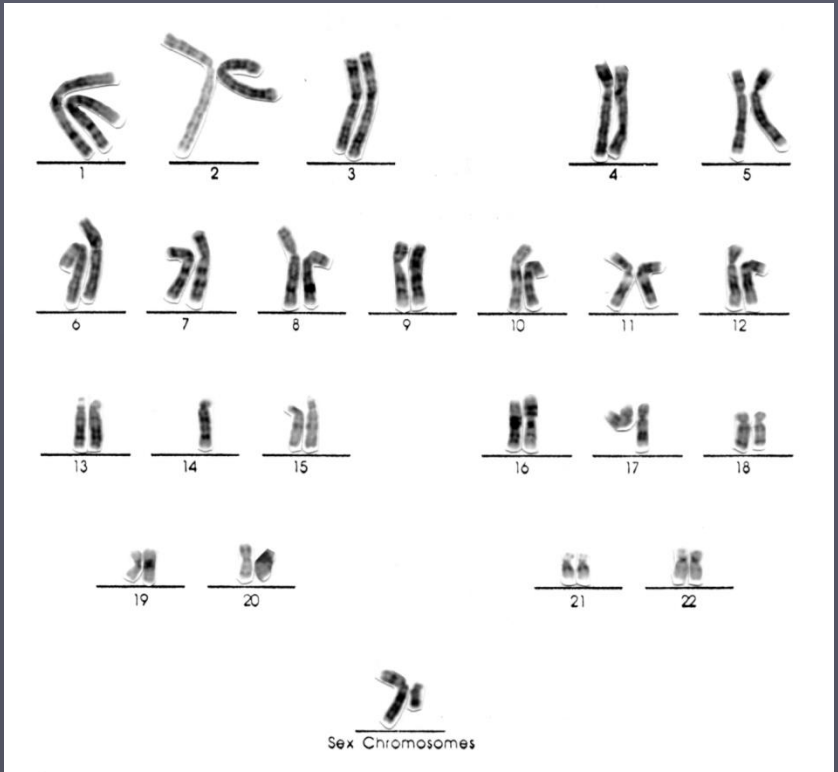
Dr. Cher-wei Liang

Step 2: MicroGIST KIT activation helped by smooth muscle



Dr. Jason Hornick

Step 3: 14q tumor suppressor: "MAX"



MAX mutations in GIST→ Cell cycle dysruption → Cell growth

nature COMMUNICATIONS

ARTICLE

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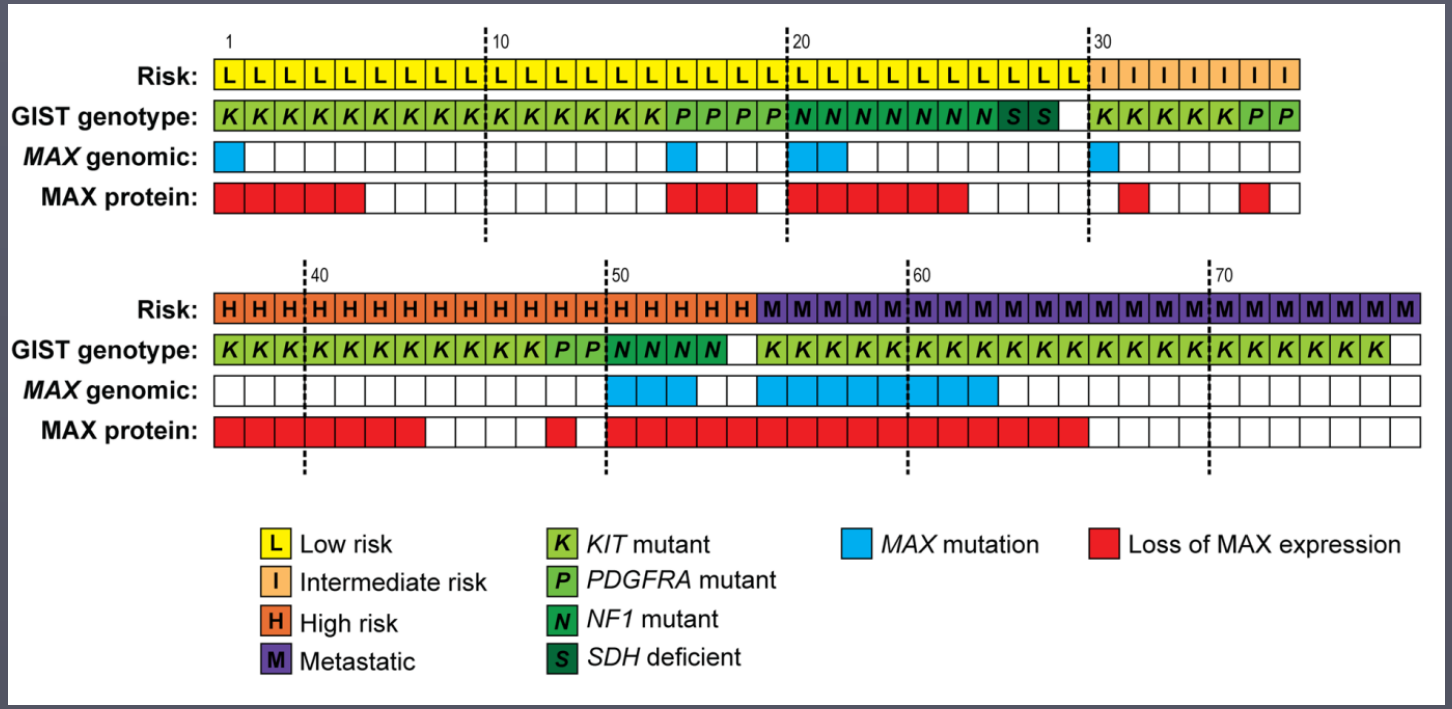
MAX inactivation is an early event in GIST development that regulates p16 and cell proliferation

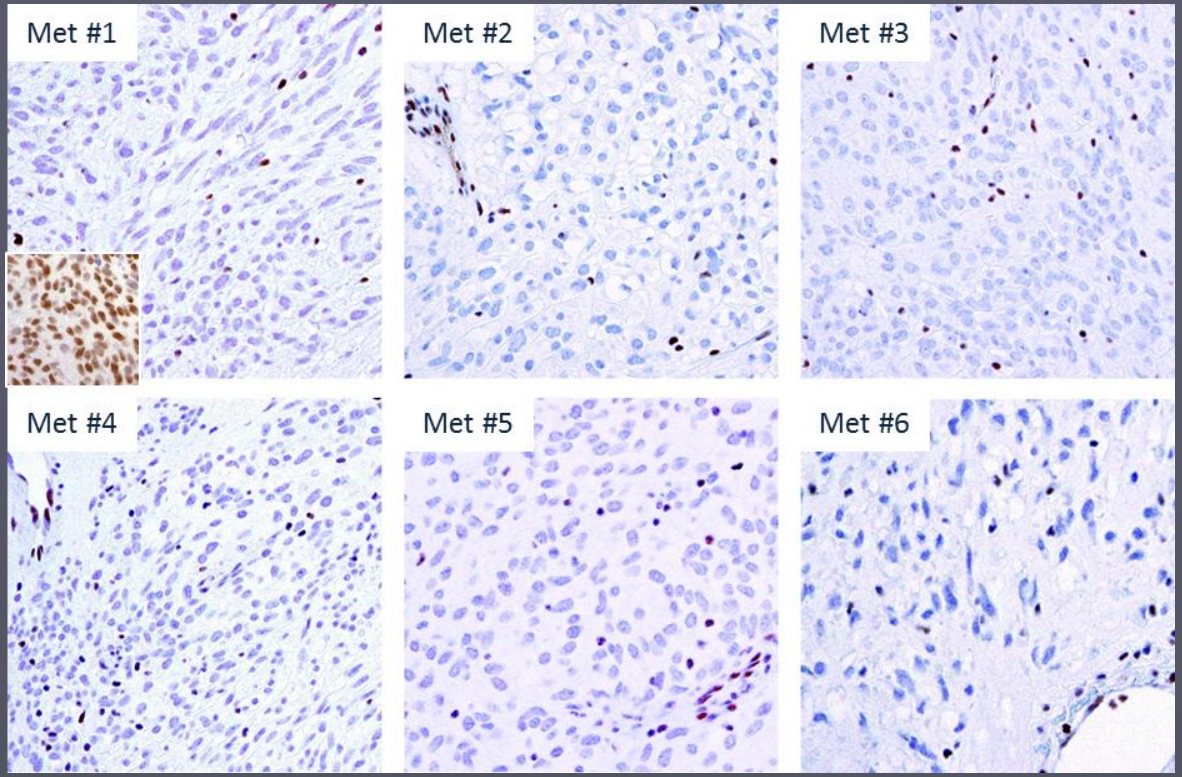
Inga-Marie Schaefer[1], Yuexiang Wang[1,†], Cher-wei Liang[1,†], Nacef Bahri[1], Anna Quattrone[1,2], Leona Doyle[1], Adrian Mariño-Enríquez[1], Alexandra Lauria[1], Meijun Zhu[1], Maria Debiec-Rychter[2], Susanne Grunewald[3], Jaclyn F. Hechtman[4], Armelle Dufresne[1], Cristina R. Antonescu[4], Carol Beadling[5], Ewa T. Sicinska[6], Matt van de Rijn[7], George D. Demetri[8], Marc Ladanyi[4], Christopher L. Corless[5], Michael C. Heinrich[9], Chandrajit P. Raut[10], Sebastian Bauer[3] & Jonathan A. Fletcher[1]

MAX inactivation in 50% of GISTs with **KIT, PDGFRA, or NF1** mutations – but NOT in SDH-deficient GISTs



Dr. Inga-Marie Schaefer – Nat Commun 2017

MAX loss in all GIST mets from a given pt



Dr. Leona Doyle – Nat Commun 2017

Step 4: 22q target inactivation

?

Step 5: Cell cycle GENOMIC mutations

Low-risk GIST (N = 16)

TP53	MDM2	CDKN2	RB1	ATM	None	%mut
0	0	0	0	0	16	0%

High-risk & Metastatic GIST (N = 32)

TP53	MDM2	CDKN2	RB1	ATM	None	%mut
5	1	18	4	1	4	>80%

Step 6: 15q target inactivation (modify the KIT oncogenic signal)

?

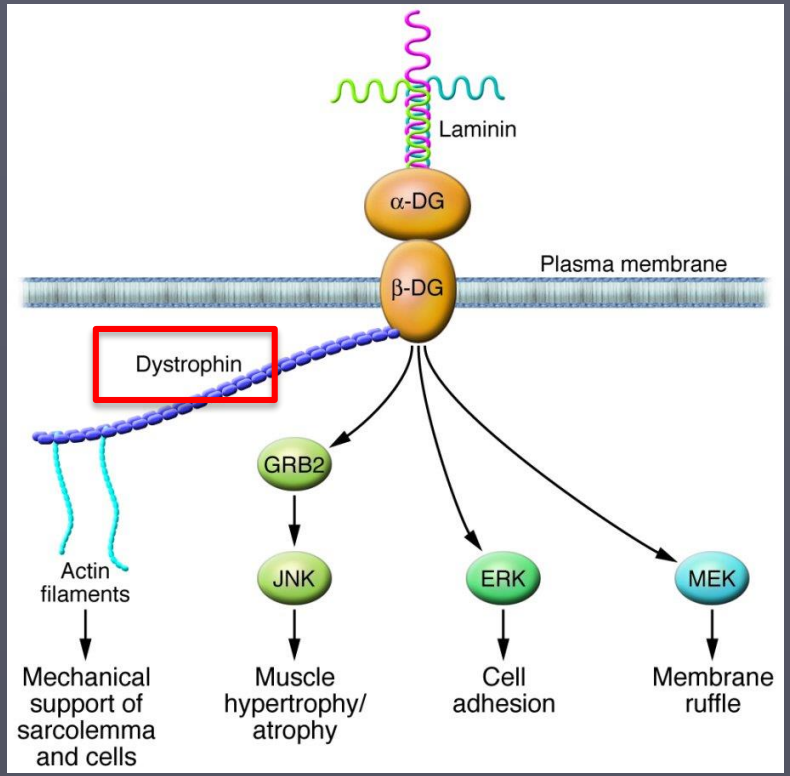
Step 7: DMD (dystrophin) inactivation

[*Duchenne muscular dystrophy gene*]

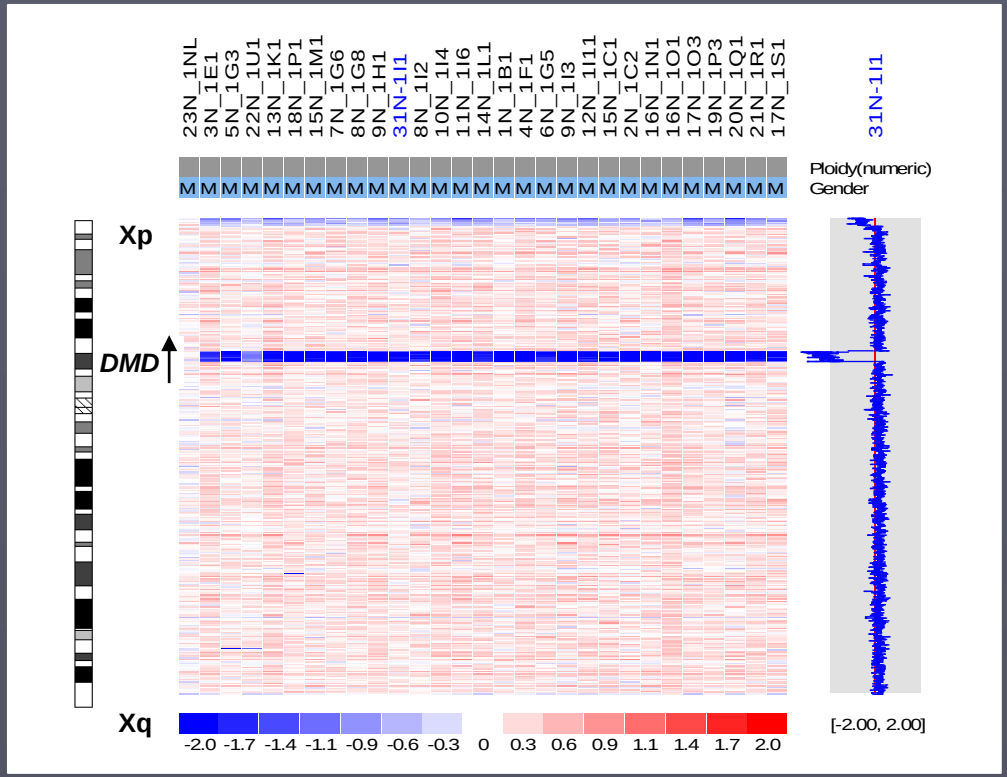
- Dystrophin expressed in low-risk GISTs
- Dystrophin inactivated in >80% of metastatic GISTs, generally by intragenic genomic deletions

Dr. Yuexiang Wang; Nature Genetics, 2014

Dystrophin communicates between cytoskeleton and extra-cellular matrix: regulates invasion, migration

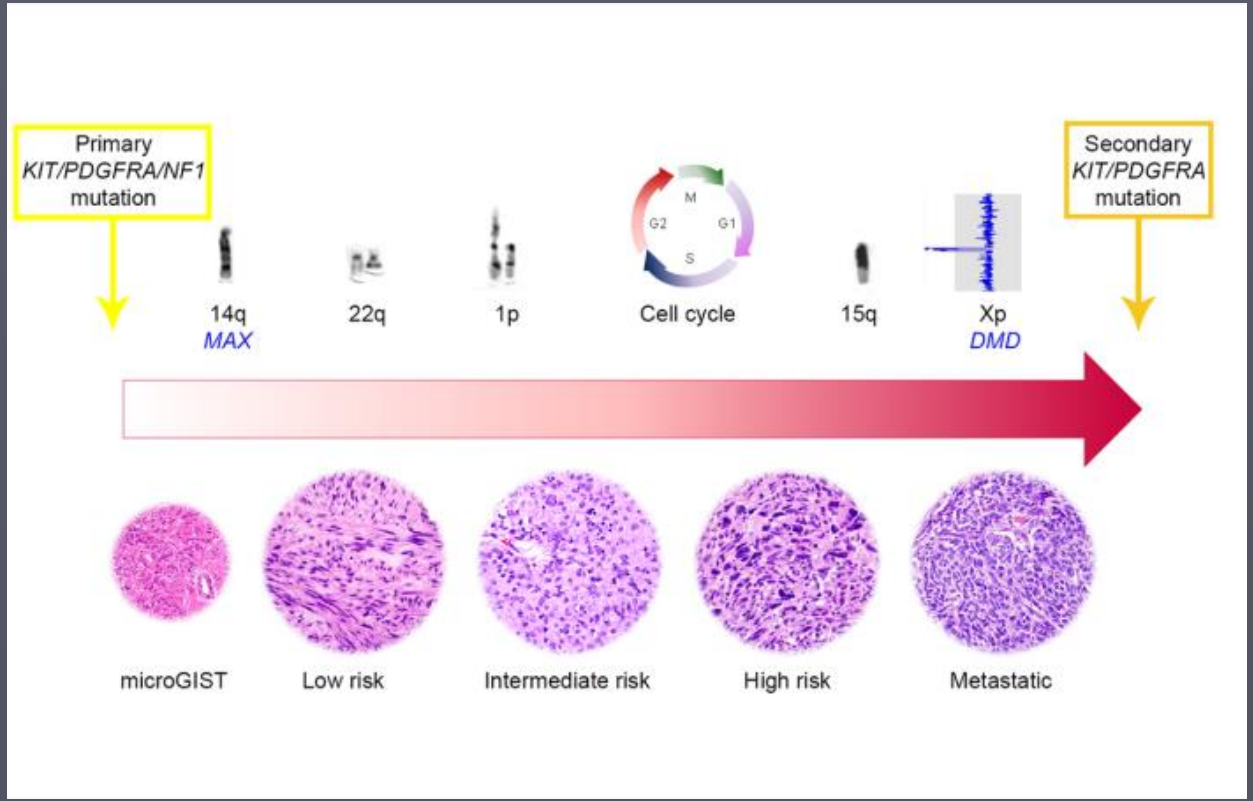


Identical *DMD* deletions in each of **28** GIST mets



Yuexiang Wang et al. Nature Genetics, 2014; 46:601-6.

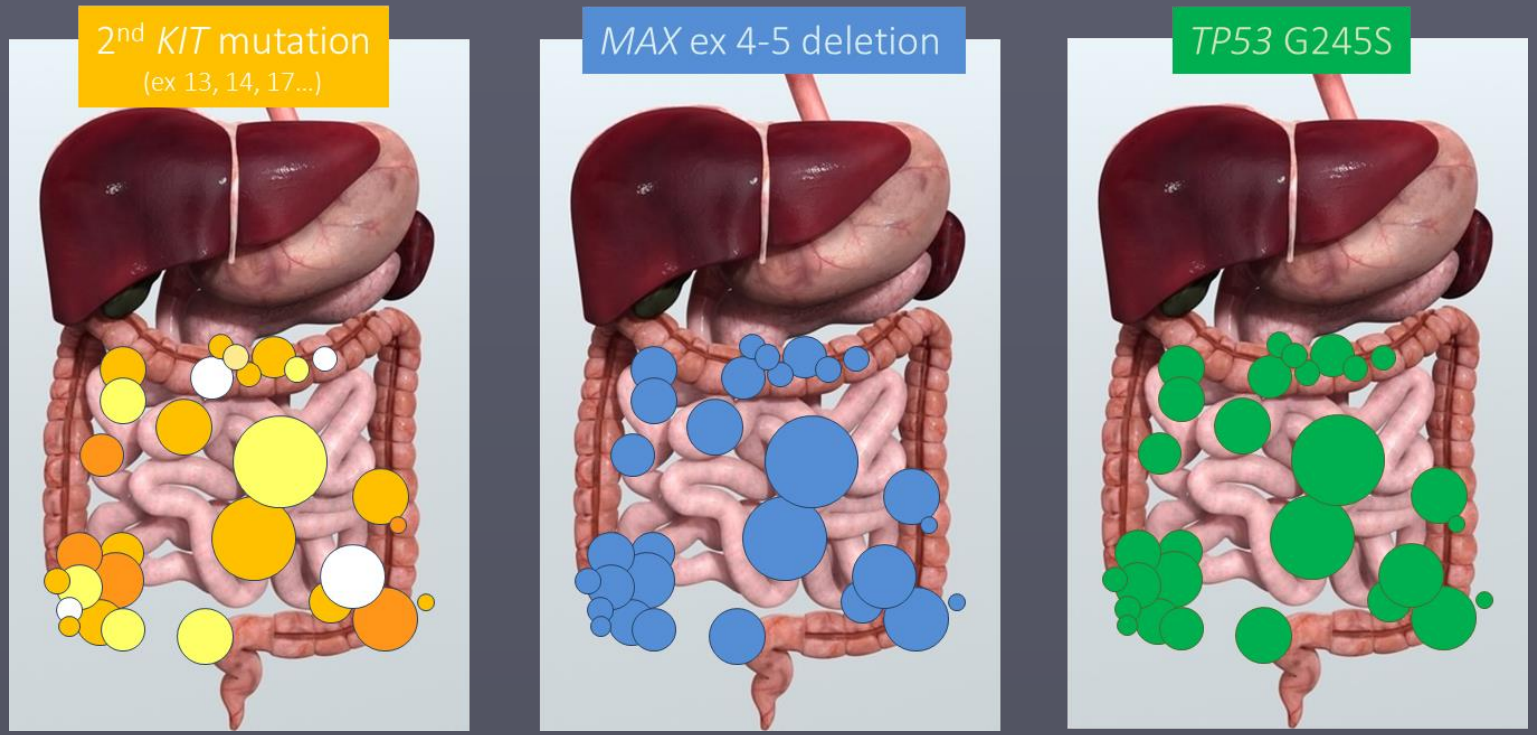
GIST Genomic Progression (KIT, PDGFRA, NF1 GISTs)



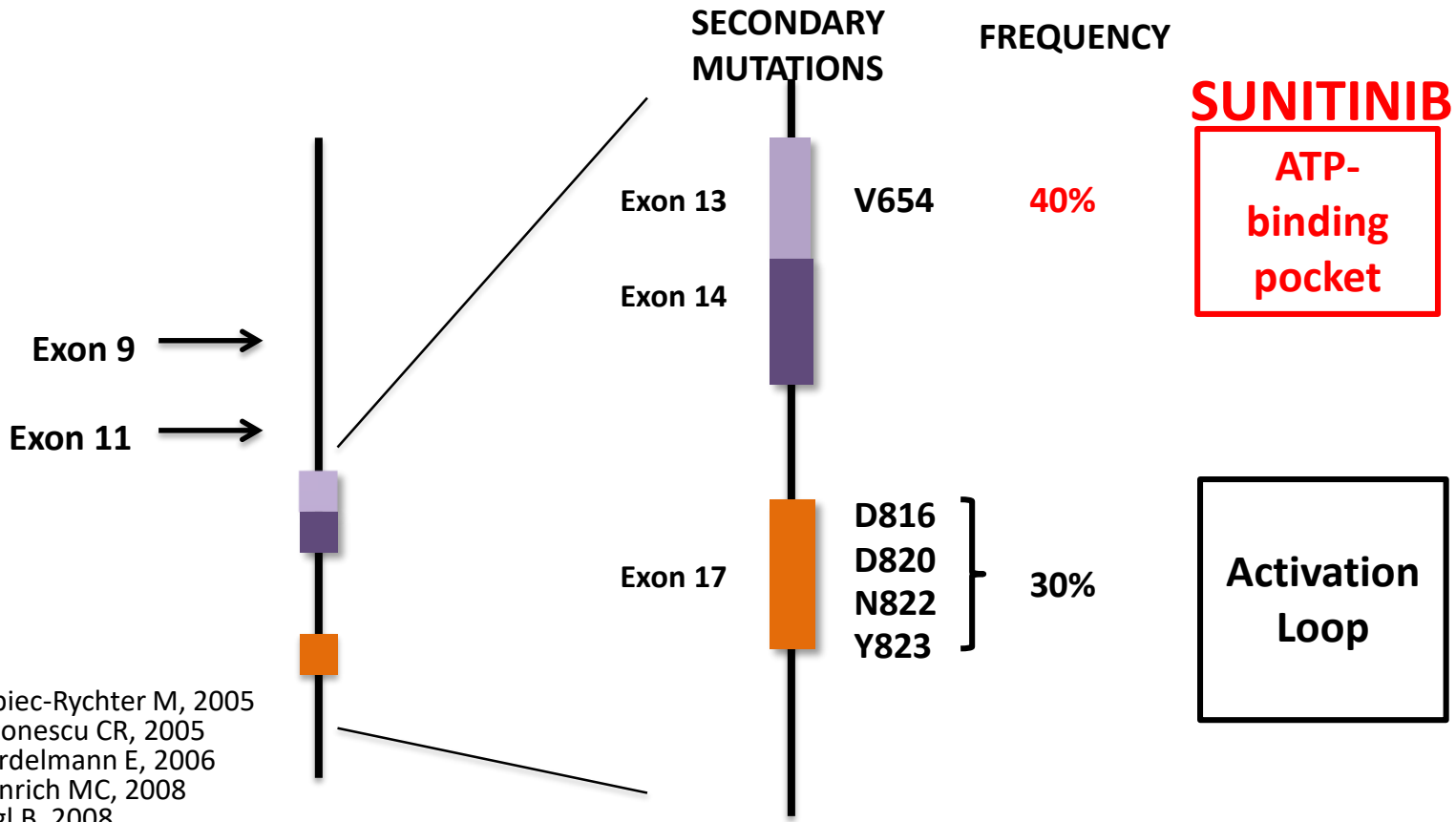
Step 8: Post-metastasis - emergence of imatinib-resistant subclones

- Imatinib-resistant 2nd KIT mutations
- Rare subclones in untreated GIST metastases
- Selected for during imatinib therapy, causing clinical progression
- 2nd KIT mutations arise independently of each other, in different metastases

29 metastases: Biologic heterogeneity & homogeneity



Secondary resistance in GIST



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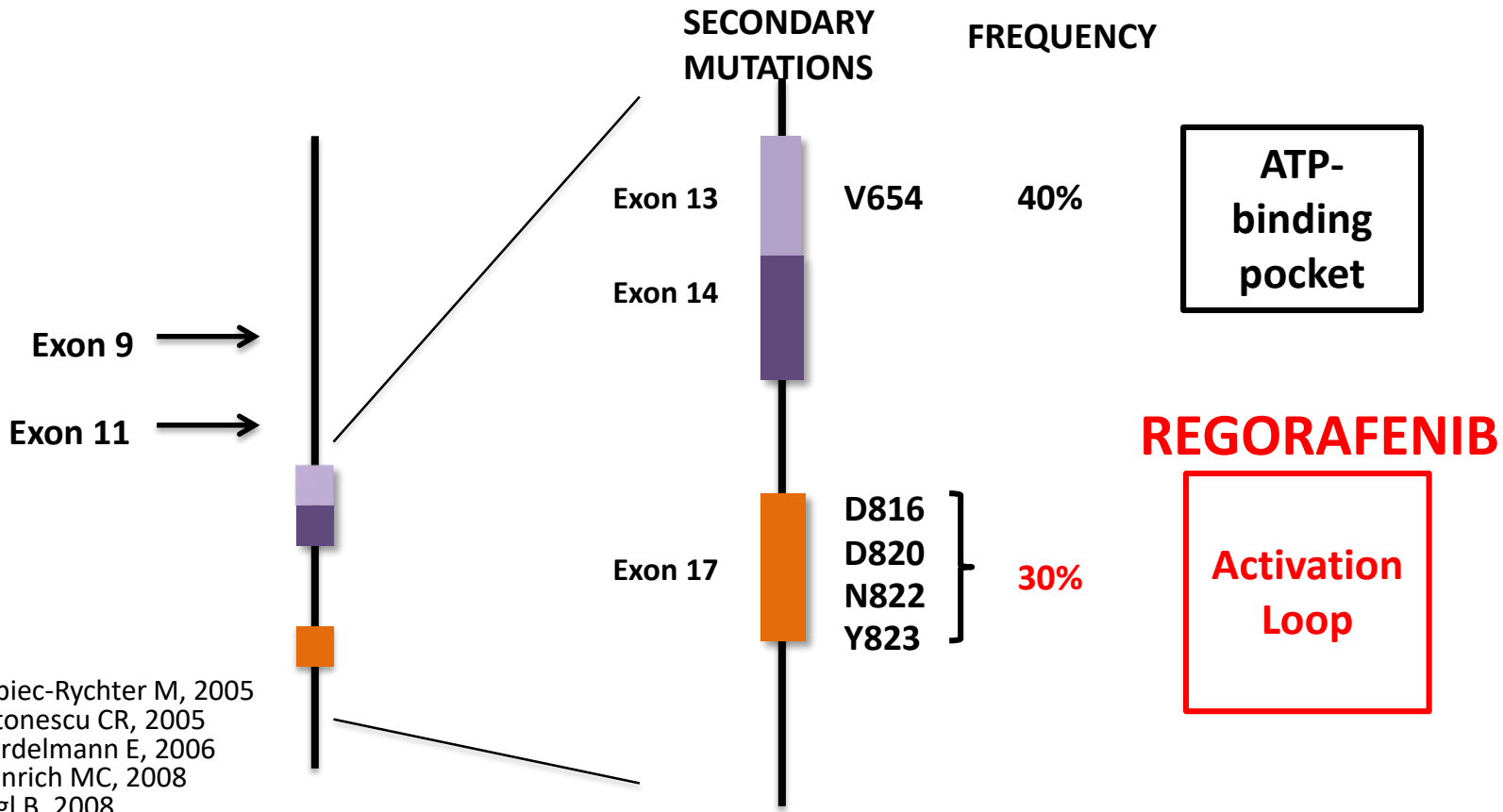
Antonescu CR, 2005
Wardelmann E, 2006
Heinrich MC, 2008
Liegl B, 2008

Secondary resistance in GIST



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Liegl B, 2008

KIT/PDGFRA inhibition strategies

- Drugs targeting particular mutations, eg PDGFRA D842V (BLU-285)
- Switch-region inhibitors (Deciphera DCC-2618)
- Alternating inhibitors for KIT exon 13 & KIT exon 17 (SuRe trial of sunitinib – regorafenib)

8 Steps of GIST Initiation/Progression

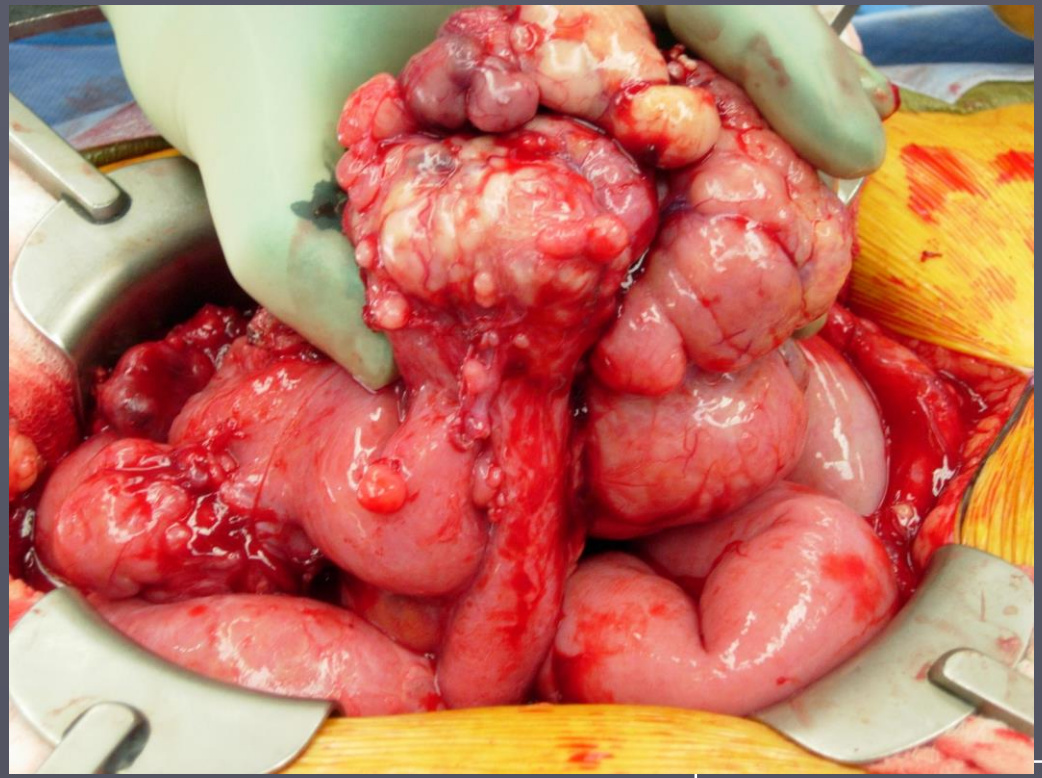
1. KIT 1st mutation: initiation, weak proliferation
2. SCF from smooth muscle: proliferation
3. MAX inactivation: p16 extinction, proliferation
4. Chr22 inactivation:
5. CDKN2A, TP53, RB1 inactivation: proliferation
6. Chr15 inactivation:
7. DMD inactivation: invasion, metastasis
8. KIT 2nd mutations: imatinib-resistance

KIT Oncogenic Signal	Cell Cycle Regulation	Cytoskeleton - ECM

Circulating GIST DNA (cfDNA): Mutation detection in **blood** samples

- Shows which imatinib-resistance KIT mutations each person has
- Many assays being developed and tested (not yet a standard approach)
- Assay sensitivity and specificity are not yet perfect

Molecular analyses of 46 nodules (52 regions sampled) After imatinib and sunitinib



Courtesy of Dr. Chandrajit Raut

Molecular analyses of 46 nodules (52 regions sampled) After imatinib and sunitinib

Specimen	*KIT* 2nd Mutation	Specimen	*KIT* 2nd Mutation	Specimen	*KIT* 2nd Mutation
1	QLP575-577H del	17	N822K	32	N822K
2	S840N	18A	N822K	33	F681L
3	S840N	18B		34	
4		18C	S840N	35A	
5		19	N822K	35B	
6	S840N	20	S840N	35C	
7	ID571-572T	21	N822K	36A	N680K
8A	S840N	22	S840N	36B	N680K
8B	S840N	23	S840N	37	
9	S840N	24	N822K	38	
10	S840N	25	N822K	39	
11	ID571-572T	26	S840N	40	
12	N822K	27	N822K	41	S840N
13		28	N822K	42	ID571-572T
14	N822K	29	N655S	43	
15	S840N	30	N822K	44	N822K
16		31	N822K	45	
				46	N822K

Yuexiang Wang

Circulating GIST DNA (cfDNA): Mutation detection in **blood** samples

- Shows which imatinib-resistance KIT mutations each person has
- Many assays being developed and tested (not yet a standard approach)
- Assay sensitivity and specificity are not yet perfect
  - GISTs seem to be "low-shedders" of DNA into blood

What do we need??

- Genome sequencing has NOT provided all the information necessary to understand GIST
- RNAseq – expression of each gene
- Epigenomics – regulation of each gene
- Proteomics – expression & activation of all proteins
- Functional genomics – knock out each gene in GIST
- ?Drug screens – test thousands of drugs

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