What’s going on in the GIST Research World?

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On my way to TEXAZZ!
What’s going on in the GIST research world?

- clinical trials
- predictive/diagnostic biomarkers
  - immunohistochemistry
  - CINSARC, aurora A
- wt/pediatric GIST
  - SDH expression/mutation
  - IGFR

- p53, cell cycle
- epigenetics, microRNA (ETV1)
- immunology
  - therapeutic (anti-KIT Ab, reg.
    T-cells, NK cells)
  - prognostic (immune infiltrate, neutrophil-lymphocyte ratio)
Pathway to Cure GIST

GIST Research Priorities

- Clinical Trials
- Primary Resistance
- Secondary Resistance
- Apoptosis
- Translational Studies
- Tissue Banks
- Pediatric GIST
- Mouse Models
- Kit Degradation
- Stable Disease
- Wildtype
- Oncogenic Signaling

The Life Raft Group
Pathway to Cure GIST
(and how to tackle the problem)

- basic biology
- predictive biomarkers
- new therapeutic targets
- new therapies
SLOW!

How did this KIT thing work again?
KIT signal transduction

- SCF
- KIT
- PDK1/2
- PI3K
- AKT/PKB
- mTOR
- p70/85S6K
- MEK1/MEK2
- MAPK p44/42
- STAT1
- STAT3
- STAT5
- JAK
- SHC
- SOS
- GRB2
- RAS
- RAF1
- Translation
- Transcription
- Survival ↑
- Growth ↑

Cell membrane
KIT signal transduction - inactive
KIT signal transduction - activation
KIT signal transduction – active

- SCF
- KIT
- PDK1/2
- PI3K
- mTOR
- p70/85S6K
- AKT/PKB
- MEK1/MEK2
- MAPK p44/42
- STAT1
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- JAK
- SHC
- GRB2
- SOS
- RAS
- RAF1
- cell membrane

- Translation
- Growth ↑
- Survival ↑

- KIT signal transduction – active
KIT signal transduction - inactivation

cell membrane

KIT

SHC
GRB2
SOS
RAS
RAF1

JAK

STAT1
STAT3
STAT5

PI3K

PDK1/2

AKT/PKB

mTOR

p70/85S6K

MAPK p44/42

MEK1/MEK2

Translation

Survival ↑

Growth ↑

Transcription

KIT signal transduction - inactivation

cell membrane

SHC
GRB2
SOS
RAS
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STAT1
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PI3K

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Translation

Survival ↑

Growth ↑

Transcription
KIT signal transduction - inactivation

Translation

PI3K

mTOR

p70/85S6K

AKT/PKB

PDK1/2

MAPK p44/42

MEK1/MEK2

RAF1

RAS

SHC

GRB2

SOS

JAK

STAT1

STAT3

STAT5

Transcription

Survival ↓

Growth ↓
Mutant KIT signal transduction

- Mutated KIT
- PDK1/2
- AKT/PKB
- mTOR
- p70/85S6K
- MEK1/MEK2
- MAPK p44/42
- STAT1
- STAT3
- STAT5
- JAK

Cell membrane

Translation

Survival ↑

Growth ↑

Survival
Mutant KIT signal transduction

- AKT/PKB
- MEK1/MEK2
- MEK1/MEK2
- MAPK p44/42
- STAT1
- STAT3
- STAT5
- JAK
- mTOR
- p70/85S6K
- PDK1/2
- PDK1/2
- mutated KIT
- cell membrane

Translation:
- Survival ↑

Growth:
- Growth ↑
Inhibition of KIT signal transduction

Kit signal transduction pathway:

- **Cell membrane**
- **Kit**
- **Imatinib (Gleevec)**

**Translation**
- mTOR
  - p70/85S6K
- AKT/PKB
- PDK1/2
- PI3K
- GRB2
- SOS
- RAS
- RAF1

**Transcription**
- STAT1
- STAT3
- STAT5
- JAK

**Growth↓**
- Survival↓
KIT signal transduction

Cell membrane

SCF

PI3K

AKT/PKB

mTOR

p70/85S6K

Translation

Survival ↑

Survival ↑

Growth ↑

SOS

GRB2

RAS

RAF1

STAT1

STAT3

STAT5

MAPK p44/42

JAK

Transcription
OK,

one more thing…
How does a cell function?
(from DNA to protein)

1. DNA
2. mRNA
3. rER
   protein assembly
4. Golgi
   protein conformation
5. done!
Pathway to Cure GIST
(and how to tackle the problem)

- basic biology
- predictive biomarkers
- new therapeutic targets
- new therapies
Basic Biology of GIST
GIST stem cells

Hypothesis:

\[ \text{KIT}^{\text{low}}\text{PDGFRA}^{\text{low}}\text{CD34}^+ \text{ ICC stem cells cause GIST resistance to TKIs} \]

- constitutively activating KIT mutations increase ICC stem cell numbers
- transformed ICC stem cells give rise to \[ \text{KIT}^{\text{low/-}} \text{ GIST-like tumors} \] - similar to those found in some long-term imatinib-treated patients
- ICC stem cells are insensitive to imatinib

Bardsley MR et al., Gastroenterology 2010
ETV1

- “transcription factor”
- highly expressed in
  - GIST
  - ICC (“interstitial cells of Cajal”)
- needed for ICC/GIST development
- regulated by KIT
- potential therapeutic target

Chi P et al., Nature 2010
Heinrich MC and Corless CL., Nature 2010 [editorial]
So... You say to yourself... What the heck is ETV1?

ETV1 is chief engineering officer "Scotty" for the starship Enterprise. KIT receptor is Captain KIRK barking orders for warp 9 tumor growth.

ETV1 (Scotty) implements the orders by making the necessary adjustments to the ship’s engines or dilithium crystals. In other words ETV1 is the component in the cell’s nucleus (the ship’s engine room) that performs the actual task of revving up the engines...that is facilitating a change in the expression of certain genes that will promote growth and survival of the GIST cell.

ETV1 is a “transcription factor.” and does it’s job in the cell nucleus. It and other proteins bind directly on the DNA of a gene to activate gene expression. KIT is a cell surface receptor that acts like a satellite dish and television set to receive growth signals from the outside and then to broadcast them into the room (the insides of the cell).

Tie up or disable Scotty (or ETV1) and then Captain Kirk’s (KIT) orders can’t be carried out. So far, our treatments (Gleevec, Sutent, Tasigna, Regorafenib, sta9090) have focused on shutting up KIRK. But we could go down to engine room instead and take out ETV1 (Scotty).
DOG1 in GIST

- diagnostic marker
- co-regulated with but not dependent on KIT
- specific target, because highly expressed
- tumor growth partially dependent on DOG1
- biochemical inhibitors with improved selectivity needed

Simon S et al., Cancer Res 2013
GIST whole genome (sequencing) studies

Goal: to identify additional mutated genes in GIST that can be targeted therapeutically or serve as biomarkers

- MDACC
  - whole genome copy number aberrations (CNA; array CGH)
  - whole genome gene expression
  - 42 GIST vs. 30 LMS

- Med. Uni. Vienna
  - whole genome CNA (SNP array)
  - whole exome sequencing focusing on target regions
  - 29 GIST (CNA), 13 exome

- OHSU
  - unbiased whole exome sequencing
  - GIST primary tumors and cell lines
  - 18 GIST patients, 4 cell lines
Biomarkers
CINSARC GIST study

- Goal: prognostic markers for clinical outcome
- CINSARC (Complexity INdex in SARcomas) signatures and tumor Genomic Index (GI)
- more sensitive in prediction recurrence than current histopathologic risk scheme
- GI index is an independent prognostic factor
- identifies poor prognosis patients in intermediate-risk group

Lagarde P et al., Clin Cancer Res 2012
free circulating tumor DNA

- free DNA (not inside a cell) circulating in the blood
- increased in cancer patients
  - dying cells inside a tumor disintegrate and release DNA
  - live tumor cells can get into bloodstream, but disintegrate there
- detected with highly sensitive techniques
  - mutated KIT as low as 0.01% (1 in every 10,000!)

complete “liquid biopsy”
  ➔ heterogeneity of tumors and metastases in GIST!
New Therapeutic Targets
ROR2 as therapeutic target in GIST

- receptor tyrosine kinase (ligand = Wnt5)
- enhances invasion in vitro (GIST and LMS)
- knockdown inhibits invasion and decreases tumor size in xenografts
- expression level correlates with outcome (high ROR2 = poor outcome)
- therapeutic target in GIST

Edris B et al., J Pathol 2012
The Notch pathways as therapeutic target in GIST

• neural function and development
  → ICC?

• cell-to-cell signaling
  • receptor on one cell
  • ligand on second cell

• cell proliferation
The DREAM complex as therapeutic target in GIST

- imatinib leads to a reversible cell cycle exit in GIST cells (tumor cell “sleep”)
- cells are not dividing or growing – BUT are metabolically active and not dead
- potential reservoir for resistant clones

- Key molecular regulator: DREAM complex

- can be targeted therapeutically to enhance apoptotic effect of imatinib

Boichuk S et al., Cancer Res 2013
Targeting the ubiquitin-proteasome machinery in GIST

- the FDA-approved proteasome inhibitor bortezomib (Velcade®) induces apoptosis in GIST cells (Bauer S et al. Cancer Res. 2010)
- new therapeutic option for GIST patients, BUT suboptimal pharmacokinetics
- second-generation proteasome inhibitors
  - drug X: FDA-approved for multiple myeloma (MM)
  - drug Y: Phase II for MM
High-throughput gene knockdown studies
siRNA/shRNA library screens

Goal: identify important survival genes in GIST besides KIT/PDGFRA
High-throughput gene knockdown studies
siRNA/shRNA library screens

genome-wide screen

- CDC37
  - crucial cofactor for KIT expression
  - interacts with oncogenic KIT
  - regulates expression and activation of KIT and downstream signaling intermediates
  - knockdown leads to KIT inhibition
  - promising target for inactivating KIT

Marino-Enriquez A et al., Oncogene 2013
New Therapies
Regorafenib (Stivarga®)

- oral multikinase inhibitor
  - KIT, PDGFR, FGFR, VEGFR2/3, TIE-2, B-RAF
- significant activity in patients with advanced GIST
- FDA-approval
- potent inhibitor of $KIT$ exon 11 mutations
- significant activity against $KIT$ exon 17 (activation loop) secondary mutations
- less active against $KIT$ exon 13 (V654A, ATP-binding pocket) mutations than SU

Serrano-Garcia C et al., ASCO 2013
Demetri GD, Lancet 2013
PI3K inhibitor GDC-0941

- significant reduction in tumor volume alone and in combination with imatinib
- higher histological response in combination treatment arm, especially apoptotic activity
- no tumor re-growth after treatment discontinuation in the combination treatment arm
- combination treatment (imatinib + GCD-0941) yields long-lasting effect (in mice!)

Grade 3 histologic response in a combination treatment

Floris G et al., Clin Cancer Res 2012
KIT-specific compounds against gatekeeper mutation (T670I)
Combined Therapies for GIST

modified after Taylor BS et al. Nat Rev Cancer 2011
Combined Therapies for GIST
Combined Therapies for GIST

modified after Taylor BS et al. Nat Rev Cancer 2011
Combined Therapies for GIST

The combination of imatinib, a PI3K inhibitor and a MAPK inhibitor can control all known GIST cell lines - while IM is unable to control GISTs with either resistant KIT mutations (5R) or those that have switched over to another oncogenic pathway (10R)

Rubin BL, personal communication
anti-KIT antibody as therapy in GIST

- TKI-resistant GIST still dependent on KIT expression and activation
- progressing disease can have several secondary resistance mutations → difficult to target with TKI
- anti-KIT mAb SR1
  - inhibits GIST cell viability
  - xenograft growth
- alternative to TKI therapy in GIST, especially in IM-resistant disease

Edris B et al., PNAS 2013
Drug compound screens

Goal: identify active compounds for the treatment of GIST

Drug compound libraries \(\rightarrow\) GIST cells

- focus on kinase inhibitors
- focus on FDA-approved drugs

Treatment in 96-well plates

Apoptosis
Cell viability

Goal: identify active compounds for the treatment of GIST
Screen has identified two major FDA-approved drug classes effective in GIST

- ~100 FDA-approved drugs tested \(\rightarrow\) drug repurposing
- *transcriptional* and *topoisomerase II* inhibitors are active in GIST cell lines and xenograft models
- clinical trials are being discussed
Summary:
What is going on in the GIST research world?

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- predictive biomarkers
- new therapeutic targets
- new therapies
Summary:
What is going on in the GIST research world?

A lot is going on in the GIST research world!!!
THANK YOU,
for your attention!

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