Novel therapeutic strategies for GIST: Targeting the tumor and treating the whole patient

GIST Patient Summit
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University of Pittsburgh School of Medicine
GISTs are caused by activating \textit{KIT} mutations

- high KIT protein expression
- \textit{KIT} gene mutations (75-85\%) → gain of function
- \textit{KIT} mutation-negative cases
  - \textit{PDGFRA} (5-7\%)
  - “wildtype” (10-15\%)

\textbf{KIT expression and KIT mutations in GIST:}
- \textit{diagnostic marker}
- \textit{therapeutic target}

Duensing A et al. Cancer Res 2004
How does imatinib induce apoptosis in GIST cells?
although imatinib is a pretty good drug, what are potential flaws that we need to overcome?

to develop new therapies, we need to know how exactly imatinib works

new therapeutic strategies

new therapies

incomplete remissions
disease recurrence
The cell
The cell
The cell

cell membrane
ribosome
nucleus
cytoplasm
The cell

cell wall
ribosome
nucleus
cytoplasm

modified from: http://www.ntz.de
The cell

- cell wall
- ribosome
- nucleus
- cytoplasm

modified from: http://www.ntz.de
Normal Function of KIT
Normal Function of KIT

Cell Membrane

SCF  SCF

KIT

PDK1/2 → PI3K

AKT/PKB

mTOR → p70/85S6K

Translation

Survival Signals ↑

Growth Signals ↑

MAPK p44/42

MEK1/MEK2

RAS

RAF1

JAK

STAT1

STAT3

STAT5

Transcription

SCF

SCF

SHC

GRB2

SOS
Normal Function of KIT

Cell Membrane

Growth Signals ⇓

Survival Signals ⇓
KIT in GIST

Cell Membrane

mutated

KIT
KIT in GIST

Cell Membrane

Mutated KIT

PDK1/2

AKT/PKB

mTOR

p70/85S6K

MEK1/MEK2

MAPK p44/42

RAS

RAF1

JAK

STAT1

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Survival Signals ↑

Growth Signals ↑

Translation

Transcription
How to stop KIT signaling in GIST?

Cell membrane

KIT

Translation

mTOR → p70/85S6K

PDK1/2 → AKT/PKB

PI3K

mTOR

Translation

Survival Signals

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Transcription

KIT

PI3K

SOS

GRB2

RAS

RAS

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MAPK p44/42

STAT1

STAT3

STAT5

JAK
How to stop KIT signaling in GIST?

Stop KIT signaling with:

- **Gleevec® (imatinib)**
- **Sutent® (sunitinib)**
- **Stivarga® (regorafenib)**

Cell membrane

Growth Signals

Survival Signals
Is inhibition of ABL by imatinib beneficial for GIST treatment?

imatinib inhibits:
• KIT
• PDGFRA
• PDGFRB
• BCR-ABL
  (chronic myeloid leukemia)
• ABL

normal ABL has various cell protective functions
ABL is present in GIST cells

To our surprise, depletion of KIT and ABL1 in GIST, we set out to dissect the molecular mechanism of ABL1 and KIT co-expression in GIST.

**Figure 1:** KIT and ABL1 are co-expressed in GIST.

- **ABL1** expression was observed in a variety of GIST cell lines, including GIST882, GIST-T1, GIST490, GIST488B, K562, SK-LMS1, and SK-UT-1.
- **KIT** expression was also detected in these samples, except for GIST48B, which is known to be KIT-negative.

**Figure 2A:** Whole cell lysates of fresh frozen human GIST samples were immunoblotted for expression of the ABL1 and KIT proteins. GIST882 cell lysates were included to compare expression levels to samples shown in (A).

**Figure 2B:** Immunohistochemical staining of a tissue microarray was performed to assess ABL1 expression in primary and metastatic GISTs. Case 3 shows high ABL1 expression in the stomach, while Case 4 displays low ABL1 expression in the small bowel.

**Figure 2C:** ABL1 and KIT expression in primary and metastatic GISTs was assessed by immunohistochemical staining of a tissue microarray. Case 3 shows high ABL1 expression in the stomach, while Case 4 displays low ABL1 expression in the small bowel.

**Figure 2D:** Depiction of the native ABL1 protein (125 kDa) and the BCR-ABL1 fusion protein (210 kDa) for expression of the ABL1 and KIT proteins. GIST882 cell lysates were included to compare expression levels to samples shown in (A).

**Figure 2E:** The above results were corroborated by biochemical analyses (Figure 2A). As expected, depletion of KIT and ABL1 in GIST cells showed a significant reduction in proliferation compared to silencing of KIT alone (Figure 2D). Moreover, we could show that combined silencing of KIT and ABL1 led to an attenuated induction of caspase 3 cleavage when compared to depletion of KIT alone. Similarly, the AKT–S6 kinase (S6K) axis was inhibited after siRNA-mediated knockdown of KIT (Supplementary Figure S1, Figure 2B).

**Figure 2F:** Inhibition of ABL1 leads to activation of AKT in GIST cells after siRNA-mediated transfection of KIT and ABL1. Knockdown of KIT (Supplementary Figure S1, Figure 2B) led to inhibition of MAPK signaling as assessed by reduced MAPK p42/p44 phosphorylation at T202/Y204. This suggests that the pro-apoptotic and anti-proliferative effect of combined silencing of KIT and ABL1 could thus be disadvantageous in the therapeutic setting.

**Figure 2G:** Taken together, these results indicate that loss of ABL1 in addition to KIT attenuates the pro-apoptotic and anti-proliferative effect of combined silencing of KIT and ABL1, which could thus be disadvantageous in the therapeutic setting.

**Figure 2H:** The above results were corroborated by biochemical analyses (Figure 2A). As expected, depletion of KIT and ABL1 in GIST cells showed a significant reduction in proliferation compared to silencing of KIT alone (Figure 2D). Moreover, we could show that combined silencing of KIT and ABL1 led to an attenuated induction of caspase 3 cleavage when compared to depletion of KIT alone.
“Inhibiting” KIT and ABL kills less GIST cells than “inhibiting” KIT alone.
ABL1 knockdown and chemical inhibition of ABL1 induce activation of AKT.

We have shown above (Figure 2E) that siRNA-mediated cyclin A isoform, facilitating entry into S phase [18, 19]. AKT thereby enabling its full activation [17]. CDK2 acts in concert with cyclin A2, the predominant mammalian kinase CDK2 has the capability to directly phosphorylate ABL1 kinase, but this effect is not attenuated by ABL1 inhibitors. Inhibition of ABL1 with GNF-2 and imatinib inhibits ABL1, whereas sunitinib and regorafenib are substantially less effective.

To corroborate these results, we inhibited PDK1 with nilotinib and CRKL phosphorylation (C) shows that imatinib inhibits ABL1 whereas sunitinib and regorafenib are substantially less effective. The majority of gastrointestinal stromal tumors is a tractable target disease. Especially imatinib, the first approved KIT inhibitor, changed the course of treatment for patients with locally advanced or metastatic GIST. However, KIT is expressed in other contexts, and modern KIT inhibitors also have adverse off-target effects. It is hence desirable to reduce the ABL1 inhibitory capacity when designing new KIT inhibitors for GIST. The BCR-ABL1 fusion oncogene is almost exclusively expressed in CML. By contrast, the native, oncogenic fusion protein BCR-ABL1 as well as the oncogenic fusion protein BCR-PDGFR as well as other receptor tyrosine kinases are expressed in a variety of solid tumors.

ABL1 inhibitors. In (A), the bands likely depict phosphorylated CRKL, a ~40 kDa protein. Band intensity was measured using LI-COR imaging software. In vivo and in vitro studies of the BCR-ABL1 fusion oncogene. By contrast, the native ABL1 protein kinase is a negative regulator of the cell division cycle [8]. Early studies have shown that the BCR-ABL1 kinase is often viewed as an oncoprotein that is crucial for stress response and growth arrest as well as progression of the cell division cycle [8].

New KIT inhibitors should have increased specificity for KIT and reduced ability to inhibit ABL. Sunitinib, regorafenib → no significant ABL inhibition. Nilotinib, dasatinib → strong ABL inhibition. BLU-285, DCC-2618
Novel therapeutic strategies in GIST

Cell Membrane

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

Survival Signals $\uparrow$

Growth Signals $\uparrow$

Mutated KIT

Translation

Transcription
Getting rid of KIT...
Stops KIT signaling in GIST!

**Translation**

- PDK1/2
- PI3K
- AKT/PKB
- mTOR
- p70/85S6K

**Growth Signals**

- MAPK p44/42
- MEK1/MEK2
- RAF1
- RAS
- SOS
- GRB2
- SHC
- JAK

**Survival Signals**

- STAT1
- STAT3
- STAT5

**Cell membrane**
How is KIT made?
(from DNA to protein)

1. DNA
2. mRNA
3. rER
   - protein assembly
4. Golgi
   - protein conformation
5. done!

transcription
(from DNA to RNA)

translation
(from RNA to protein)
GISTs are sensitive to transcriptional inhibitors
Mithramycin A:
 killing GIST cells by inhibiting KIT transcription

**Figure 2.** The transcriptional inhibitor mithramycin A (MMA) and the topoisomerase II inhibitor mitoxantrone (MXN) effectively induce time-dependent GIST cell apoptosis and cell-cycle arrest. A and B, dose-dependent effect of MMA (A) and MXN (B) on apoptosis (left) and cell viability (right) of GIST882 and GIST430 cells as measured by luminescence-based assays (mean ± SE). \( P < 0.05 \) in comparison with control. C and D, immunoblot analysis (C) for markers of apoptosis and cell-cycle regulation and TUNEL assay (D) in GIST cells after treatment with DMSO or 0.1 mol/L MMA for the indicated times. E, immunoblot analysis for markers of apoptosis and cell-cycle regulation in GIST cells after treatment with DMSO or MXN at indicated concentrations for 72 hours or with MXN at the indicated times. F, bright field image of GIST430 cells treated with DMSO or MXN (5 mol/L) for 72 hours.
Mithramycin A analogs: same activity, but better toxicity profile

collaboration with EntreChem, Ovieda, Spain

Jessica Rausch - unpublished
GISTs are sensitive to transcriptional inhibitors

Boichuk S et al., Cancer Research 2014
Second generation inhibitors of the 26S proteosome

<table>
<thead>
<tr>
<th>Bortezomib (Velcade)</th>
<th>Carfilzomib (Kyprolis, PR-171)</th>
<th>Ixazomib (MLN-9708)</th>
<th>Delanzomib (CEP-18770)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millennium</td>
<td>Onyx Pharmaceuticals</td>
<td>Millennium</td>
<td>Cephalon</td>
</tr>
<tr>
<td>26S (reversible)</td>
<td>26S (irreversible)</td>
<td>26S (reversible)</td>
<td>26S (reversible)</td>
</tr>
<tr>
<td>i.v.</td>
<td>i.v.</td>
<td>oral</td>
<td>oral</td>
</tr>
<tr>
<td>FDA-approved</td>
<td>FDA-approved</td>
<td>FDA-approved</td>
<td>Phase II/III (multiple</td>
</tr>
<tr>
<td>(multiple myeloma,</td>
<td>(multiple myeloma)</td>
<td>(multiple myeloma)</td>
<td>myeloma)</td>
</tr>
<tr>
<td>mantle cell lymphoma)</td>
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</tbody>
</table>
Inhibitors of the 26S proteasome inhibit KIT transcription similar to bortezomib

IC50:
- Bortezomib: 15 nM (10 nM)
- Delanzomib: 20 nM (11 nM)
- Ixazomib: 90 nM (50 nM)
- Carfilzomib: 500 nM (130 nM)

- second-generation 26S proteasome inhibitors are effective in GIST
- have the same mechanism of action as bortezomib
- planning clinical trial through NCI CTEP

Rausch JL et al, CTOS 2014
How is KIT made?
(from DNA to protein)

3.1 Genes and c-kit receptors

Human genetic material is made up of about 30,000 different genes. Each of these genes is present in the DNA (substance that contains the genetic information) in every cell of the body. Genes make sure that the cells form specific types of proteins. Some of these proteins communicate with other cells or genes. Cells use receptors (like aerials), as well as other elements, to help them receive messages sent out by the proteins. The receptors are constructed according to plans provided by the relevant gene in its own cell. The c-kit gene, for example, contains a kind of operating instructions which tell the cell how c-kit receptors are to be produced.
Inhibition of translation leads to loss of KIT and effectively kills GIST cells

**Homoharringtononine** (Synribo®)

- inhibitor of protein translation
- FDA-approved for treatment of imatinib-resistant chronic myeloid leukemia
development of KIT kinase inhibitors to target GIST should include reducing their ability to inhibit the ABL kinase

eliminating KIT protein expression via
- inhibition of DNA transcription or
- inhibition of protein translation

are promising treatment strategies for TKI-resistant GIST
I have CHEMO BRAIN!
What’s your excuse?
To Do:

• Buy fresh lime for the fiesta tonight
• pick up the cat’s medicine at the vet’s
• Buy a new bike helmet for Jr.
“Chemo-brain”
(Chemotherapy-related cognitive dysfunction)

- first described in the 1980s:
  "Chemotherapy is associated with measurable decrements in neuropsychological test performance" (Silberfarb et al.)
- Prevalence: ~15-80% depending on study
- what is affected?
  - verbal memory, working memory, visual-motor processing speed
- usually mild, not progressive impairments
- often confounded by
  - age, education, IQ, pre-morbid neurologic impairment, depression, anxiety fatigue, substance use
Life after diagnosis and treatment of cancer in adulthood

In addition to prevention, diagnosis, and treatment phases, the NCI designates survivorship as a distinct phase of the cancer control continuum. Evidence that particular psycho-social and behavioral experiences are more pronounced in some periods of survivorship than others leads us to propose three periods within the survivorship phase: reentry, early survivorship, and long-term survivorship. Figure 2 displays these periods and several experiences relevant to each. Although distinct periods of survivorship have some precedent in the literature (e.g., Gotay & Muraoka, 1998; Mullan, 1985), our conceptualization includes no sharp boundaries between periods, but rather fluidity in transition from one period to the next. Meant as a heuristic for considering variation along what can be a long course of survivorship, Figure 2 includes experiences commonly documented in a proportion of cancer survivors during each period. Important caveats are that some sequelae are experienced by a minority of survivors, and additional longitudinal research is needed to specify their trajectories.

The Reentry Period

The reentry period (Mullan, 1985), wherein one makes the psychosocial transition from "cancer patient" to "person with a history of cancer," typically spans the point from completion of major cancer treatments, which can vary from a few weeks to more than 1 year, through the next several months. Health care professionals often do little to prepare patients for the reentry period, which can contribute to cancer survivors and intimate others holding unrealistically lofty expectations for rapid recovery (IOM, 2006; Janz et al., 2008; Marcus et al., 2002) and being surprised by their feelings as treatment ends. As physician Elizabeth McKinley (2000) wrote, "I thought I would feel happy about finally reaching the end of treatment, but instead, I was sobbing... Instead of joyous, I felt lonely, abandoned, and terrified. This was the rocky beginning of cancer survivorship for me" (p. 479).

Stanton A, Rowland JH, Ganz PA, American Psychologist, 70, 159-174
What causes chemo-brain?  
(And why does not everyone get it?)

- widely varying hypothesized mechanisms
- direct neurotoxicity and cell death
- white matter degradation
- pro-inflammatory cytokines (“the immune system”)
- estrogen suppression – hormone therapy
- stress?
So, chemo-brain is real. But…

Why do many patients still have normal neuropsychological testing scores?

Where is the impairment?!
1-back>0-back

2-back>0-back

3-back>0-back

Chemotherapy-treated Twin Twin A

Non-cancer Twin Twin B

Ferguson RJ et al., Journal of Clinical Oncology, 2007
The chemo-brain (has to) work harder to get things done.

What are the consequences?

social life, family, employment...

And what to do about it?!
MAAT – Memory & Attention Adaptation Training

4 treatment components

- Education and “memory failure reattribution”
- Self-awareness training
- Self-regulation and stress management
- Cognitive Compensatory strategies

**MAAT**

**CRCD**
- Biological Triggers; Alterations in Brain Structure and Function
- Daily Task Performance Difficulty
- Perceptions of Disparity Between Cognitive Demands and Cognitive Abilities
- Distress Responding
- Misattribution of Distress-Related Cognitive Problems to CRCD (confirmatory experience)

Exacerbates CRCD
It works!

### Cognitive Impairments

**Neurocognitive Processing Speed**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-Treatment</th>
<th>2-Month Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAAT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Supportive Therapy</strong></td>
<td></td>
<td></td>
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</tbody>
</table>

### Quality of Life

1. Rate how satisfied you were in general with the treatment program

<table>
<thead>
<tr>
<th></th>
<th>Post Treatment</th>
<th>2-Month Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supportive Therapy</strong></td>
<td>5.5 (2.3)</td>
<td>5.7 (2.0)</td>
</tr>
<tr>
<td><strong>MAAT</strong></td>
<td>6.9 (0.8)</td>
<td>6.9 (1.1)</td>
</tr>
</tbody>
</table>

2. Rate how helpful the treatment program was with *improving* problems of attention and memory

<table>
<thead>
<tr>
<th></th>
<th>Post Treatment</th>
<th>2-Month Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supportive Therapy</strong></td>
<td>3.2 (2.5)</td>
<td>3.3 (2.1)</td>
</tr>
<tr>
<td><strong>MAAT</strong></td>
<td>5.5 (1.5)</td>
<td>5.4 (1.6)</td>
</tr>
</tbody>
</table>

3. Rate how helpful the treatment program was with helping you *compensate for and handle* memory or attention problems when they arise

<table>
<thead>
<tr>
<th></th>
<th>Post Treatment</th>
<th>2-Month Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supportive Therapy</strong></td>
<td>4.8 (2.0)</td>
<td>4.7 (2.7)</td>
</tr>
<tr>
<td><strong>MAAT</strong></td>
<td>6.2 (1.4)</td>
<td>6.3 (1.7)</td>
</tr>
</tbody>
</table>
Nothing is known...

... about cognitive dysfunction in patients on long-term tyrosine kinase inhibitor therapy.

Like you!
A web-based survey of cognitive dysfunction and other patient-reported outcomes in people with GIST

Dr. Robert J. Ferguson, PhD
Biobehavioral Oncology Program
UPMC Hillman Cancer Center
University of Pittsburgh School of Medicine

Dr. Dana Bovbjerg, PhD
Director, Biobehavioral Oncology Program
UPMC Hillman Cancer Center
University of Pittsburgh School of Medicine

Dr. Beth E. Snitz, PhD
Department of Neurology
University of Pittsburgh School of Medicine
What were the 3 items that you were assigned to remember?
What is our survey about?
What will we ask?

1. some basic questions:
   - basic demographics
   - basic clinical and treatment history

2. standardized questionnaires asking about:
   - general health
   - perceived cognitive impairments
   - fatigue
   - sleep disturbance
   - emotional distress – depression
   - pain
Important to know...

- validated, widely used questionnaires with strong psychometric properties

- PROMIS (patient-reported outcomes measurement information system)
  - library for health care researchers to measure health with valid and reliable questionnaires about symptoms, function and quality of life
  - access for researchers through credentialed registration process
  - limits spread of questionnaires and copyright violations

- standardized reference populations
  - normed on thousands of patients and healthy individuals

- numerical rating scale
Important to know...

- completely anonymous
- no personal or private health information is being asked
- nobody can be identified
WE WANT YOU
to directly participate in our research!
Now! (And later...)

- take our survey (once it goes live...)
- will be posted on GSI listserv and GSI Facebook page

- let us know if we missed something
- fill out form in your conference package
- leave with me or the conference organizers (Ginger, Marina...)
- or: email me with suggestions: aduensin@pitt.edu
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- Joseph Siino

Cleveland Clinic
- Brian Rubin

numerous private donors
Conclusions

NEED TO REVISE TO INCLUDE ALL OF THE PREVIOUS CHEMO BRAIN

• MAAT appears to be more effective than other cognitive behavioral therapy.

• MAAT likely has positive sustained effects on quality of life (less anxiety about cognitive failures).

• It can readily be delivered electronically with high survivor satisfaction.
WE WANT YOU
ABL is a survival factor in GIST cells

- new KIT inhibitors for GIST should have increased specificity for KIT and reduced ability to inhibit ABL
- sunitinib, regorafenib → no significant ABL inhibition
- nilotinib, dasatinib → strong ABL inhibition
- BLU-285, DCC-2618