Autophagy inhibition overcomes sorafenib resistance in $CTNNB1$ mutant S45F desmoid tumors

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Philadelphia, PA
Desmoid Tumors

- Rare and understudied
- No randomized clinical trials
- Standard treatments remain uncertain
- Treatment strategies: challenging for physicians
- Targeted therapies: being used currently
Sorafenib: clinical biology

- Multikinase inhibitor
- Clinical antitumor activity: phase III clinical trial
### Sorafenib: side effects

**Table 2. Clinical benefit, radiological responses, and toxicities associated with sorafenib (Cont’d)**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Location</th>
<th>Initial symptoms</th>
<th>Clinical benefit on sorafenib</th>
<th>Best RECIST response to sorafenib</th>
<th>Best T2 MRI signal change (from baseline)*</th>
<th>Visual approximation of &quot;darker than muscle.&quot;</th>
<th>Sorafenib toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (P)</td>
<td>Abd/RP</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td>−30%</td>
<td>Diarrhea (gr 2) related to surgery.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (P)</td>
<td>CW/T</td>
<td>Dyspnea on exertion, sharp chest pain, edema, impending cardiopulmonary collapse.</td>
<td>Significant decrease in symptoms within 1 mo. Complete symptom resolution in 3 months.</td>
<td>−19%</td>
<td>HF (gr 2), diarrhea (gr 1), fatigue (gr 1).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 (P)</td>
<td>Abd/RP</td>
<td>Severe abd pain</td>
<td>Worse on treatment</td>
<td>−9%</td>
<td>None. Off therapy for toxicity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 (P)</td>
<td>Abd/RP</td>
<td>Pain from hydronephrosis</td>
<td>Pain relief with ureteral stent.</td>
<td>−8%</td>
<td>BF, fatigue, rash, dry skin.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 (F)</td>
<td>Abd/RP</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td>−8%</td>
<td>Fatigue, nausea, vomiting (grade 1, resolved)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (F)</td>
<td>Abd/RP</td>
<td>Intermittent abdominal pain</td>
<td>Worsening of symptoms</td>
<td>−7%</td>
<td>Unilateral blurry vision.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 (P)</td>
<td>Abd/RP</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td>−3%</td>
<td>Severe. Off therapy for toxicity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 (P)</td>
<td>Abd/RP</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td>+11%</td>
<td>HF, diarrhea, nausea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23 (P)</td>
<td>Abd/RP</td>
<td>Abdominal pain, distension</td>
<td>Worsening pain. POD and death.</td>
<td>+17%</td>
<td>Dry hands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 (P)</td>
<td>Abd/RP</td>
<td>Intermittent abd fullness</td>
<td>No improvement in symptoms.</td>
<td>+18%</td>
<td>H&amp;F syndrome, hypertension. Off therapy for no benefit.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 (F)</td>
<td>Abd/RP</td>
<td>Abdominal fullness</td>
<td>Abdominal fullness</td>
<td>No scan</td>
<td>HTN: Gr 3. Off therapy for toxicity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 (P)</td>
<td>RP/PS</td>
<td>Significant back pain</td>
<td>Immediate relief within 1 week.</td>
<td>No scan</td>
<td>Rash, dry skin. N.V. grade 1.</td>
<td></td>
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</tr>
</tbody>
</table>

*Methods section for calculation of signal decrease.

**Abbreviations:** mo, months; L-ROM, Limited range of motion; LE, lower extremity; UE, upper extremity; CW/T, Chest wall or trunk; H&N, head and neck; Abd/FP, abdomen or retroperitoneum; Abd, abdominal; RP/PS, retroperitoneal and paraspinal; GI includes: nausea, vomiting and/or diarrhea; HF, Hand-Foot syndrome.

*Decrease in T2 signal as estimated by a single radiologist blinded to treatment. FAP: familial adenomatous polyposis. (F) First line sorafenib, (P) Priortherapies.

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**Gounder et al., Clin Cancer Research, 2011**
Sorafenib: responses

Gounder et al., Clin Cancer Research, 2011
Study goal

To further elucidate molecular mechanisms of sorafenib action and resistance utilizing DT cell strain models
Materials and Methods

- 78 desmoid cell strains isolated at OSU confirmed as DT cells via CTNNB1 genotyping of cells and original tumor

- Proliferation, migration, invasion assays, WB, FACS (cell cycle/apoptosis)
S45F mutation confers sorafenib tolerance even at lower doses

DT cells were treated with Sorafenib for 14 days
Sorafenib mediates decreased DT migration, but not in S45Fs!!
Sorafenib mediates decreased DT invasion, but not in S45Fs!!
S45F mutation blocks sorafenib-mediated apoptosis
S45F mutation blocks sorafenib-mediated apoptosis

Effects of sorafenib on cell apoptosis measured by IncuCyte® ZOOM
Steps in autophagy

1. Phagophore
2. Autophagosome
3. Lysosome
Sorafenib resistance in S45F-mutated desmoid cell strain is associated with activation of autophagy

**Sorafenib (1μM)** - +

D168 (T41A)

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<tr>
<th>Sorafenib (1μM)</th>
<th>-</th>
<th>+</th>
</tr>
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<tr>
<td><strong>LC3-II</strong></td>
<td></td>
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<td><strong>GAPDH</strong></td>
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1.00 1.20 RFU

D23 (S45F)

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1.00 4.76 RFU
Assessing autophagy inhibition

2. Bafilomycin
DT cells were treated with Sorafenib and bafilomycin for 14 days

CTNNB1 S45F-mutated cells are dependent on autophagy for survival
Assessing autophagy inhibition
CTNNB1 S45F-mutated cells are dependent on autophagy for survival
Conclusions

- S45F-mutated DTs are more tolerant to low, clinically relevant doses of sorafenib
- S45F-mutated DT cells: dependent on autophagy as a survival mechanism

Does autophagy dependence underlie S45F-mutated DT cells tolerance to sorafenib?
Future directions

Clinical trial: sorafenib combined with other autophagy inhibitors (HCQ)—beneficial to S45F-mutated desmoid patients?
## Acknowledgements

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- Gonzalo Lopez
- Lucia Casadei

**The James**

**THE DESMOID TUMOR RESEARCH FOUNDATION**

**THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER**
CTNNB1 S45F-mutated cells are dependent on autophagy for survival
Combination of sorafenib and hydroxychloroquine has no effects on cell viability on T41A mutated DTs in vitro.
Combination of sorafenib and hydroxychloroquine enhances the anti-proliferative effects on S45F mutated DTs in vitro