A Phase 1, Dose Escalation Study of Intravenous TK216 in Patients with Relapsed or Refractory Ewing Sarcoma

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TK216 is a Novel, First-in-Class And Only-in-Class Small Molecule Inhibitor of ETS-Family Oncoproteins

- Pharmacological inhibitors of EWS-FLI1 were identified using surface plasmon resonance screening by Jeffrey Toretsky and Aykut Üren at Georgetown University & Lombardi Comprehensive Cancer Center.

- Research compound YK-4-279 showed activity against Ewing sarcoma based on interaction with FLI1 domain of EWS-FLI1, and subsequent activity in a larger panel of tumors that rely on ETS family members.
  - ETS family members: FLI1, ERG, ETV1, ETV4, ETV5, ETV6, ETS1, ETS2, & SPIB
  - These share high homology in the DNA binding domain and surrounding regions.

- Clinical candidate TK216, developed by Oncternal Therapeutics, is being tested in a Phase 1 study for patients with Ewing sarcoma.
In Ewing sarcoma, EWS-FLI1 interacts with transcription factors, splicing proteins and histone regulators causing:
- Activated oncogenes
- Inhibited tumor suppressors
- Abnormal RNA transcription
- Abnormal RNA splicing

TK216 was designed to disrupt binding and activation resulting in:
- Decreased oncogene expression
- Increased tumor suppressor function
- Improved transcription and splicing
- Apoptotic cell death

Erkizan 2009 Nature Medicine
Hong 2013 Oncotarget
EWS-FLI1 inhibitor YK-4-279 Reduces Rat Xenograft Tumors

• Nude rats injected orthotopically (tibia) with ES cells
• Dosing initiated when tumors reached 2.5 to 3 cm³
  - Mean TGI = 95%, p<.001

Hong et al Oncotarget 2014
• EWS-FLI1 inhibitor YK-4-279 and vincristine (VCR) have synergistic effects:
  - YK+VCR induced G2-M arrest, increased cyclin B1, and decreased EWS-FLI1–mediated generation of microtubule-associated proteins → increased microtubule depolymerization by VCR, enhanced apoptosis
  - YK+VCR reduced tumor burden and increased survival in mice bearing A4573 tumor xenografts

Zoellner, et. al., 2017 Science Signaling
Protocol TK216-01
Phase 1 Dose Escalation in Ewing Sarcoma Patients

- Recurrent/Refractory ES, 32 patients treated so far
- 3+3 Design, Continuous IV dosing
- 3 Parts:
  1. **Dose Escalation** (Completed)
     - 7 day infusion via portable pump
     - Followed by 14 days rest period (21-day cycles)
     - N = 21 evaluable patients
  2. **Schedule Escalation** (Ongoing)
     - 10 days and more infusion
     - Followed by mandatory 14-day rest period in first cycle; flexible thereafter
     - Option to add vincristine, increase dose and/or infusion length from Cycle 3
  3. **Expansion cohort** (planned N=18)
     - TK216 at RP2D + vincristine + BM support from Cycle 1

- 7 clinical sites
  - MD Anderson
  - MSKCC
  - UCLA
  - Children's Hospital Colorado
  - Duke
  - Texas Children’s
  - Cleveland Clinic

- Objectives
  - Safety/tolerability, pharmacokinetics
  - DLTs, MTD and Phase 2 dose selection
  - Biomarker assessment
  - Antitumor effects: ORR (RECIST), survival
Treatment Schema:

Cohorts 1 to 6
7 d infusion, 14 d recovery

Cohorts 7 & 8*
10 d infusion, 14 d recovery
Vincristine option cycle 3+

*Preclinical data showed greater efficacy with longer drug exposure

Cohort 9
14 d infusion, 14 d recovery
Vincristine option cycle 3+
Patient Demographics:

<table>
<thead>
<tr>
<th>N= 32,  N (%)</th>
<th>Age: Mean (Median)</th>
<th>Range</th>
<th>Race: White: 23 (72%)</th>
<th>Asian: 6 (19%)</th>
<th>Hispanic: 1 (3%)</th>
<th>Not Specified: 2 (6%)</th>
<th>Gender: Male: 21 (66%)</th>
<th>Female: 11 (34%)</th>
<th>Disease Status at Diagnosis: Localized: 19 (59%)</th>
<th>Metastatic: 13 (41%)</th>
<th>Time from Diagnosis to Enrollment: Mean (Median): 52.9 (44.5)</th>
<th>Range: 5.4 to 191.7</th>
<th># Lines of Prior Systemic Therapy*: Mean (Median): 3.8 (4)</th>
<th>Range: 1 to 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>N= 32,  N (%)</td>
<td>Mean (Median)</td>
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<td>White: 23 (72%)</td>
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<td>Mean (Median): 3.8 (4)</td>
<td>Range: 1 to 9</td>
</tr>
</tbody>
</table>

*includes all reported systemic therapy patient received for metastatic disease and initial therapies
Protocol TK216
Dose Escalation And MTD For 7 Day Schedule

7 day TK216 CIV treatment in mg/m²/day, 14 day recovery

MTD for 7 day infusion = 220 mg/m²/day

Cohort 1-4:
• No DLTs
• No myelosuppression

Cohort 5:
• DLT (Neutropenia)
• Variable and manageable myelosuppression
• Early signs of activity with stabilization of disease

Cohort 6:
• No DLTs
Protocol TK216
Schedule Escalation: 7 to 14 days with initial 14 day break (Ongoing)

Cohort 7, N=3

220 mmd x10 d

220 mmd x14 d

No DLT

DLT

2 DLT: Neutropenic fever

Cohort 8, N=4

200 mmd x10 d

No DLT

200 mmd x14 d

Cohort 9

175 mmd x10 d

• When the TK216 220mg/m²/day CIV was increased from 7 to 10 days DLTs occurred
• MTD 10 day infusion: 200 mg/m²/day CIV
• Currently 200mg/m²/day CIV for 14 days has been well-tolerated
• Vincristine (VCR) allowed starting in cycle 3

mmd = Starting dose in mg/m²/day TK216 infusion
The majority of TK216-related AEs were transient grade 1/2 events due to marrow suppression. Most common grade 3/4 related events included leukopenia/neutropenia, neutropenic fever, anemia, thrombocytopenia. 16 patients reported SAEs: Most were associated with their underlying cancer; 8 pts reported SAEs considered possibly related to TK216 incl: transient neutropenia/febrile neutropenia and 1 event each of influenza-like illness and periorbital cellulitis.

### Adverse Events Considered Related to TK216 (>10%)

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
<th>Cohort 5</th>
<th>Cohort 6</th>
<th>Cohort 7</th>
<th>Cohort 8</th>
<th>Cohort 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Subjects N=31</td>
<td>Dose 18 mg/m² 7 Days N=3</td>
<td>Dose 36 mg/m² 7 Days N=3</td>
<td>Dose 72 mg/m² 7 Days N=3</td>
<td>Dose 144 mg/m² 7 Days N=3</td>
<td>Dose 200 mg/m² 10 Days N=4</td>
<td>Dose 200 mg/m² 14 Days N=2</td>
<td>Dose 220 mg/m² 7 Days N=3</td>
<td>Dose 220 mg/m² 10 Days N=3</td>
</tr>
<tr>
<td># of pts with an event</td>
<td>22 (71.0%)</td>
<td>2 (66.7%)</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
<td>4 (100%)</td>
<td>1 (50.0%)</td>
<td>1 (33.3%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>10 (32.3%)</td>
<td>0</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>1 (33.3%)</td>
<td>2 (50.0%)</td>
<td>0</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>6 (19.4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (25.0%)</td>
<td>0</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>6 (19.4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (25.0%)</td>
<td>0</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>6 (19.4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>5 (16.1%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>9 (29.0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>2 (50.0%)</td>
<td>1 (50.0%)</td>
<td>0</td>
<td>3 (100.0%)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>6 (19.4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>1 (25.0%)</td>
<td>0</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (16.1%)</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (50.0%)</td>
<td>0</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>5 (16.1%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (25.0%)</td>
<td>1 (50.0%)</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>4 (12.9%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>2 (28.6%)</td>
</tr>
</tbody>
</table>
Half-life is relatively long (>6 h) with dose proportional increase in concentrations between Cohorts 6 and 5.

Additional PK testing is underway but exposure & half-life appear to increase further with longer infusions.

Preclinical data suggest that TK216 levels in the 0.2-0.4 μM range were effective at tumor killing in vitro and plasma levels in the low μM were associated with efficacy in animal tumor model. These appear achievable with current doses.
Case Report: Patient History

• 3/2015 presentation with Ewing sarcoma of clavicle with multiple pulmonary metastases
• Initial therapy as per AEWS 1221, regimen A: interval dose compression with cycles of VDC and IE
  - 6/2015 resection with positive margins, followed by adjuvant RT to 50.4 Gy
  - 1/2016 completion planned systemic therapy
• 3/2016 completion whole lung RT to 15 Gy
• 8/2016 biopsy proven metastatic pulmonary nodule
• 9/2016 to 7/2017 received 13 cycles of irinotecan/temozolomide
• 8/2017 to 5/2018 received bevacizumab
• 9/2017 added pazopanib, frequently interrupted because of severe mucosal toxicity
• 10/2018 CT chest 3 new nodules and resumed pazopanib
• 2/2019 CT chest demonstrated unequivocal progressive disease, at least one pulm. nodule >1 cm
Positive for the following somatic alterations in the investigational panel:

- FGFR1 (NM_001174067) exon13 p.N577K (c.1731C>G)
- POLE (NM_006231) exon45 p.A2056T (c.6166G>A)
- EWSR1 (NM_013986) - FLI1 (NM_002017) fusion: t(11;22)(q24.3;q12.2)(chr11:g.128660704::chr22:g.29684397)
  - Note: The EWSR1 - FLI1 fusion involves EWSR1 exons 1 - 8 and FLI1 exons 6 -9. The fusion is predicted to be in frame.
Case Report: Treatment With TK216

• In Mar 2019, patient enrolled onto the TK216 Phl clinical trial

• Dose level 9, TK216 200 mg/m²/day as a continuous infusion for 14 days

• 4/30/2019 CT chest after 2 cycles of TK216 almost complete resolution of all pulmonary nodules

• No hematopoietic toxicity

• As per protocol vincristine added to cycle 3 et seq., and interval between cycles shortened

• 6/10/2019 CT chest sustained response

• 7/16/2019 CT chest sustained response: one persistent subpleural nodule

• 8/23/2019 Excised last remaining residual nodule 0.7 x 0.5 x 0.5 cm
TK216 Dose level 9

LLL nodule 2/22/2019 13 mm target lesion

LLL nodule 4/30/2019 resolved
TK216 Dose level 9

RLL 7.5 mm nodule 2/22/2019

RLL nodule 4/30/2019 almost resolved
TK216 Dose level 9

Lingula 2/22/2019

Lingula 4/30/2019
TK216 Dose level 9

LLL nodule 2/22/2019 2 mm

LLL nodule 4/30/2019 4 mm
TK216 Dose level 9

RLL 6 mm lesion 2/22/2019

RLL 3 mm lesion 4/30/2019
7/26/2019 residual subpleural nodule
Patient remains NED

• Imaging studies 22 October 2019 remain NED
• Patient continues on investigational agent 8 months from study entry
• Patient remains NED 2 months following resection of residual nodule
Summary

• **First in human study of TK216:** A novel agent directed against the ETS family of oncoproteins which is highly expressed in Ewing sarcoma as well as other malignancies

• **Safety:** Overall well-tolerated, with dose limiting toxicity of manageable myelosuppression. There was minimal marrow suppression at the current schedule of 200 mg/m²/day TK216 for up to 14 days +/- VCR

• **Pharmacokinetics:** Dose proportional increase with increasing dose and half-life of >6 hours at the 7 day CIV MTD of 220 mg/m²/day and data suggest longer half-lives with greater duration of infusions

• **Efficacy:** Major tumor regression observed in a heavily pretreated patient at the current dose schedule with the longest TK216 exposure of 14 days. The patient remains with no evidence of disease on treatment after surgical complete remission.
  - Note: Tumor regression was observed after 2 cycles of TK216 alone -- before VCR added to regimen with cycle 3

• **Study Status:** Enrollment of final dose-finding cohort is ongoing, with plans to begin Expansion Cohort if no DLTs

• **Conclusion:** The early clinical activity of TK216 along with evolving preclinical data suggests that this agent warrants further examination in Ewing sarcoma as well as in a variety of other cancer indications
On first looking into Chapman's Homer
by John Keats

• Then I felt like some watcher of the skies
• When a new planet swims into his ken
• Or like stout Cortez when with eagle eyes
• He star'd at the Pacific – and his men
• Look'd at each other with a wild surmise –
• Silent, upon a peak in Darien