A phase 1, dose-escalation, safety, pharmacokinetic, pharmacodynamic study of thioureidobutyronitrile, a novel p53 targeted therapy, in patients with advanced solid tumors

Geoffrey Shapiro[a], Jeffrey G. Supko[b], Daniel Cho[c], John Hilton[a], Matthew Hadfield[a], Solida Pruitt-Thompson[a], Eveline Bordoli-Trachsel[a], Nela Zvereva[b], Andrew Wolanski[a], Aya Sato-DiLorenzo[a], Susan Gotthardt[c], Edward A. Fox[a], Sigitas Jonas Verselis[a], Ashok Kumar[d], Sylvia A. Holden[d], Siya Ram[d], Karima Chafai-Fadela[d], Kimberly Harding[d], Krishna Menon[d]

[a]Dana-Farber Cancer Institute, Boston, MA; [b]Massachusetts General Hospital, Boston, MA; [c]Beth Israel Deaconess Medical Center, Boston, MA; [d]Cellceutix Corporation, Beverly, MA

INTRODUCTION

Thioureidobutyronitrile, Kevetrin™, has demonstrated anti-tumor activity in several wild type and mutant p53 xenografts without evidence of genotoxicity. Kevetrin induces cell cycle arrest and apoptosis through activation and stabilization of wild type p53, resulting in increased expression of target genes p21 and PUMA (Kumar *et al.* 2011). Kevetrin also alters processivity of MDM2, and induces monoubiquitination of wild type p53, enhancing its stability (Kumar *et al.* 2012).

Activation of wild type p53 by Kevetrin is non-genotoxic and induces apoptosis in tumors. p53 function is lost or inactive in about 50% of human cancers. Various stresses induce mutations and decrease the fidelity funnel through a p53-MDM2 node in the signaling pathway. This node is very important for preventing the cancer. p53 is a tumor-suppressor protein that induces cell cycle arrest and apoptosis in response to genotoxic stress. p53 is regulated primarily by the ubiquitin ligase MDM2, which binds to p53 leading to proteasomal degradation.

Mutant p53 is an array of mutant proteins with oncogenic properties varying among patients. Mutant p53 proteins increase proliferation and chemoresistance in cancer cells. Depletion of mutant p53 in tumors induces apoptosis. A drug that activates wild type p53 and degrades mutant p53 is an excellent candidate for cancer treatment. Kevetrin induces degradation of oncogenic mutant p53 and induces apoptosis (Kumar *et al.* 2012). Kevetrin therefore has the unique ability to target tumors with both wild type and mutant p53 in a wide range of tumor types.

Based on these data, a Phase 1 study is being conducted at Harvard Cancer Centers :
Dana-Farber Cancer Institute, Beth Israel Deaconess Medical Center, and Massachusetts General Hospital

PHASE 1 STUDY

Harvard Cancer Centers

Dana-Farber Cancer Institute, Beth Israel Deaconess Medical Center, Massachusetts General Hospital
ClinicalTrials.gov Identifier: NCT01664000

Objectives:

- Evaluate the safety, tolerability, maximum tolerated dose (MTD) and determine recommended Phase 2 dose
- Characterize pharmacokinetic (PK) profiles
- Evaluate preliminary evidence of anti-tumor activity
- Explore potential biomarkers of tumor response

Methods:

- 1-hour intravenous infusion once weekly for 3 weeks in 28-day cycles
- Starting dose was 10 mg/m^2.
- Dose escalation in a 3+3 design, groups of 3-6 patients evaluated for toxicity at each dose level.
- Dose escalation is based upon the number and intensity of adverse events in cycle 1.
- The total number of subjects planned is up to 40.
- Once the MTD is established, up to 12 additional subjects may be enrolled at the MTD dose level.
- PK is characterized for the first and last doses given in cycle 1.
- Anti-tumor activity by RECIST 1.1 criteria and serum tumor markers will be assessed.
- p53 status of tumors of selected patients will be determined.

PD Biomarker:

p21 expression in peripheral blood mononuclear cells.

Key Eligibility Criteria:

Adults with refractory locally advanced / metastatic solid tumors, acceptable liver, kidney function, and hematological status.

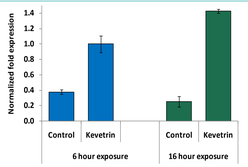
Demographics of Trial Participants

Cohort	Dose (mg/m^2)	Age	Sex	Race	Ethnicity	Cycles Completed	Tumor
0	10	45	Female	White	Non-Hispanic	2	Metastatic squamous cell carcinoma with metastases to the liver
0	10	55	Male	White	Non-Hispanic	1	Metastatic squamous cell carcinoma with metastases to the lungs
0	10	80	Female	White	Non-Hispanic	7	Endometrium stroma sarcoma
1	20	73	Female	White	Non-Hispanic	1	Metastatic ovarian cancer
1	20	76	Female	White	Non-Hispanic	1	Metastatic melanoma with metastases to the lungs, left thigh, and large bowel
1	20	71	Female	White	Non-Hispanic	1	Metastatic endometrial cancer with metastases to the thorax and abdomen
1	20	69	Female	Asian	Non-Hispanic	2	Small cell esophageal carcinoma with metastases to the liver
2	30	55	Male	White	Non-Hispanic	2	Prostate cancer
2	30	62	Male	White	Non-Hispanic	1	Bladder cancer
2	30	69	Female	White	Non-Hispanic	2	Pancreatic cancer with liver lesions

Results:

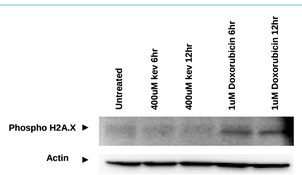
- The Phase 1 trial began November 7, 2012.
- A total of 10 patients have been enrolled to date.
- Cohorts 0, 1 and 2 have been completed without DLT.
- The MTD has not yet been reached; the trial is ongoing.

Expression levels of p21 in lymphocytes is a bio-marker for Kevetrin



Real time PCR analysis of p21 gene expression. Mice were injected with 200mg/kg IP of Kevetrin. Blood samples were collected at 6 or 16 hrs after injection. Lymphocytes were isolated and RNA was extracted. Expression levels of p21 were measured using qPCR (Kumar *et al.* 2011).

Kevetrin did NOT induce the phosphorylation of H2A.X indicating that Kevetrin is non-genotoxic

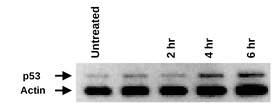


Western blot analysis of extracts from A549 cells untreated or treated with 400μM of Kevetrin or 1 μM of Doxorubicin at indicated times using phospho H2A.X rabbit mAb (Kumar *et al.* 2011).

BACKGROUND

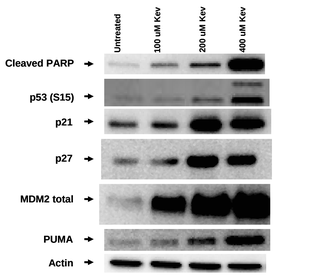
wild type p53

Kevetrin induced the p53 activation in A549 (wt p53)



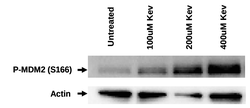
Western blot analysis of extract from A549 cells untreated or treated with 400 μM Kevetrin for different time using p53 mAb.

Kevetrin induced apoptosis and transcriptional targets in p53 in A549 (wt p53)



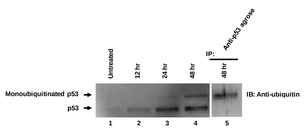
Western blot analysis of extract from A549 cells untreated or treated with increasing concentration of Kevetrin(48 hr) using different mAb.

Kevetrin induced phosphorylation of MDM2 (S166)



Western blot analysis of extracts from A549 cells untreated or treated with Kevetrin for 48hr using phospho-MDM2(S166) rabbit mAb.

Kevetrin acts on E3 processivity of MDM2 and monoubiquitinates p53

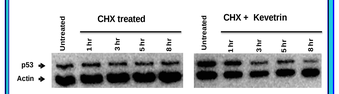


Western blot analysis of extracts of A549 cells untreated or treated with 400μM Kevetrin for indicated time using p53 antibody (lane 1-4). For lane 5, extract from Kevetrin treated for 48 hr was immunoprecipitated with p53 antibody. Ubiquitinated p53 was detected by immunoblotting with anti-ubiquitin antibody.

Kumar et al. 2011

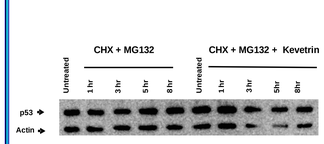
mutant p53

Kevetrin induced degradation of mutant p53 in MDA-MB-231



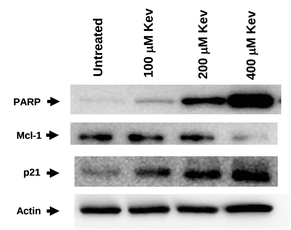
Western blot analysis of p53 Western blot analysis of extract from A549 cells untreated or treated with either cycloheximide (CHX) or Kevetrin and Cycloheximide (CHX).

Kevetrin induced destabilization of mutant p53 is rescued by proteasome inhibition



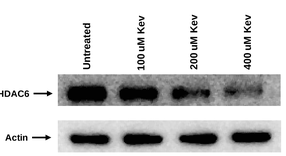
Western blot analysis of p53 Western blot analysis of extract from A549 cells treated with cycloheximide(CHX) and either proteasome inhibitor MG132 or Kevetrin and MG132.

Kevetrin induced apoptosis in MDA-MB-231 (mutant p53)



Western blot analysis of extract from MDA-MB-231 cells untreated or treated with increasing concentration of Kevetrin(48 hr) using different mAb.

Kevetrin down regulates HDAC6

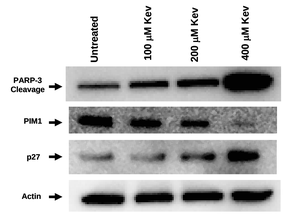


Western blot analysis of extract from MDA-MB-231 cells untreated or treated with increasing concentration of Kevetrin (48 hr) using HDAC6 mAb.

Kumar et al. 2012

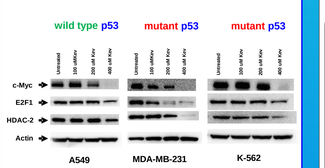
mutant p53

Kevetrin induced apoptosis and cell cycle arrest in K-562 (mutant p53)



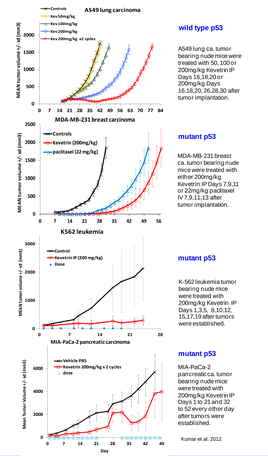
Western blot analysis of extract from K-562 cells untreated or treated with increasing concentration of Kevetrin (48 hr) using different mAb.

Kevetrin induced other signaling pathways for increasing apoptosis in A549, MDA-MB-231 and K-562 cancer cell lines



Western blot analysis of extract from A549, MDA-MB-231 and K-562 cells untreated or treated with increasing concentration of Kevetrin(48 hr) using different mAb.

Kevetrin has potent anti-tumor activity in xenograft models with varied p53 status



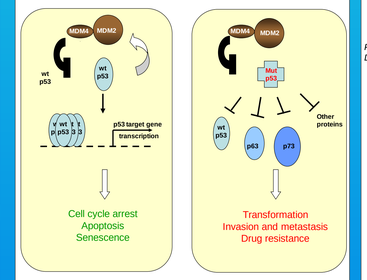
References:

Kumar *et al.* (2011) Cancer Research 71: 4470
Kumar *et al.* (2012) Cancer Research 72: 2874

CONCLUSIONS

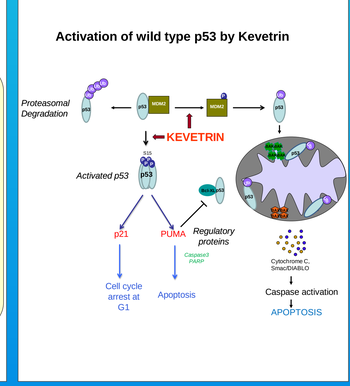
- **p53 induction by Kevetrin is non-genotoxic**
- **Tumors harboring both mutant and wild type p53 can be treated with Kevetrin**
- **Kevetrin induced apoptotic cell death in wild type p53 and mutant p53 cells through p53 apoptotic pathways**
- **Kevetrin stabilized p53 and induced p53 transcriptional targets in wild type p53**
- **Inhibition of MDM2 by Kevetrin induces transcriptional independent apoptosis**
- **Kevetrin induced degradation of mutant p53 mediated by reactivation of MDM2 E3 ligase**
- **Kevetrin is a potent anti-tumor agent**
- **Expression of p21 in lymphocytes is a biomarker for Kevetrin**
- **Kevetrin is in a Phase 1 clinical trial; 3 cohorts have been completed without DLT**

Representation of p53 pathways

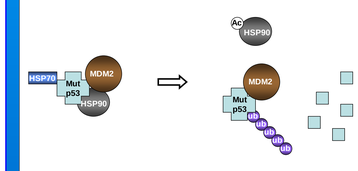


Adapted from Goh *et al.* J Pathol (2011) 116-126

Activation of wild type p53 by Kevetrin

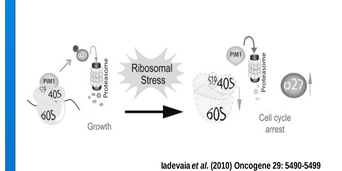


Degradation of mutant p53 by Kevetrin



Adapted from Li *et al.* (2011) Cell Death and Differentiation 18: 1904-1913

Model of the relationship between ribosomal stress and PIM1



Iadevaia *et al.* (2010) Oncogene 29: 5490-5499

For further information

Please contact Cellceutix Corporation at 978-236-8717 info@cellceutix.com
More information on this and related projects can be obtained at www.cellceutix.com