

BACKGROUND

TLN-232 is a synthetic cyclic heptapeptide with demonstrated *in vitro* and *in vivo* anti-proliferative effects in multiple cancers. Its cancer activity is mediated via a novel mechanism that induces apoptosis in tumor cells and which is thought to be mediated by the nuclear translocation of the M2 isoform of pyruvate kinase (M2PK), a newly established tumor target.

A total of four clinical studies (two Phase I and two Phase II trials) have been conducted with this compound. Until the present study, the dosing regimen comprised of a four hour intravenous infusion. The rationale for the new route of administration (i.e. continuous IV) was based on animal studies showing that continuous exposure was more effective than intermittent exposure, regardless of the route of administration. Furthermore, extended treatment of 14 versus 7 days or 28 versus 14 days resulted in greater tumor inhibition or longer term survival. These *in vivo* findings were consistent with the findings observed in *in vitro* pharmacology experiments where prolonged interaction between cancer cells and TLN-232 were required in order for the compound to induce apoptosis.

The rationale for testing TLN-232 in renal cancer was based on the known over-expression of the molecular target, M2PK. In addition, immunohistochemistry data on the presence of M2PK in kidney cancer samples indicated a strong expression of M2PK when compared to normal tissues.

We report the final data of this study.

OBJECTIVES

Primary Objective:

- To evaluate the efficacy of TLN-232 as a monotherapy in patients with metastatic kidney cancer, using RECIST criteria to assess tumor response

Secondary Objectives:

- Safety (through clinical and biological evaluations)
- Other efficacy parameters (progression-free survival rate, time to progression and overall survival, PET scans, biomarkers)
- Pharmacokinetic (PK) characteristics
- Quality of life
- Biological modulation (through potential blood and/or urine biomarkers including M2PK)

METHODS

The study was an open-label, single arm, safety and efficacy study. Each patient received multiple 28-day cycles, consisting of 21 days of TLN-232 continuous IV infusion at 0.48mg/kg/day followed by a 7-day rest period. The daily dose was based on the dose used in two previous studies. Treatment cycles were repeated unless presence of disease progression or unacceptable toxicity.

Main Inclusion Criteria:

- Patients with histologically confirmed stage IV kidney clear cell carcinoma
- Patients with progressive disease after receiving a previous systemic therapy, including at least one line of standard of care defined as immunotherapy and/or anti-angiogenic therapy
- Patient with progressive disease confirmed by 2 CT scans or MRI performed within the past 6 months
- Patients with measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques or as >10 mm with spiral CT scan
- Patients >18 years of age
- Patients with ECOG performance status ≤ 2 (Karnofsky 60%)
- Patients with normal organ and marrow function

Main Exclusion Criteria:

- Patients who have received any known medical treatment targeting cancer within 4 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier
- Patients with known brain metastases

References:

- Stetak A., Csermely P., Ulrich A., Kéri G. Physical and functional interactions between protein tyrosine phosphatase α, PI 3-kinase and PKCδ. *Biochem Biophys Res Commun.* 2001; 288: 564-572.
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- Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 2008; 452: 181-186.
- Oremek G.M., Teigelkamp S., Kramer W., Eigenbrodt E., Usadel K-H. The Pyruvate kinase isoenzyme Tumor M2 (Tu M2-PK) as a tumor marker for Renal Carcinoma. *Anticancer Research.* 1999; 19: 2599-2602.

RESULTS

A total of 10 patients were enrolled at a single site in France between March and October 2007.

Table 1: Study Treatment Summary

Patient	Date of 1 st Dose	Date of Last Dose	Last Dose Day, Cycle	Reason for Termination
001	12-Mar-2007	26-Mar-2007	D15, C1	SAE
002	21-May-2007	25-Jul-2007	D10, C3	PD
003	09-Jul-2007	20-Aug-2007	D15, C2	PD
004	16-Jul-2007	20-Feb-2008	D21, C5	PD
005	23-Jul-2007	18-Feb-2008	D21, C5	PD
006	20-Aug-2007	10-Sep-2007	D22, C1	Consent withdrawal
007	03-Sep-2007	12-Nov-2007	D15, C3	Clinical progression
008	10-Sep-2007	26-Nov-2007	D22, C3	PD
009	17-Sep-2007	19-Oct-2007	D5, C2	PD
010	05 Nov-2007	05-Nov-2007	D1, C1	SAE

SAE = Serious Adverse Event; PD = Progressive Disease; ET = Early Termination

Efficacy Results:

Three (3) patients completed 3 cycles and were evaluated (RECIST): one patient showed disease progression and two patients (**#004** and **#005**) were stable. Those two patients began their cycle 4 therapy following an unforeseen treatment interruption of 78 days due to delays in the regulatory approval process for the trial extension beyond cycle 3 (Protocol Amendment). Both patients were found to have disease progression at the time they started cycle 4. Decision was taken to continue therapy and to repeat scans at the end of cycle 5. This second radiological evaluation (CT scan and bone scintigraphy) confirmed disease progression and both patients were discontinued from the study.

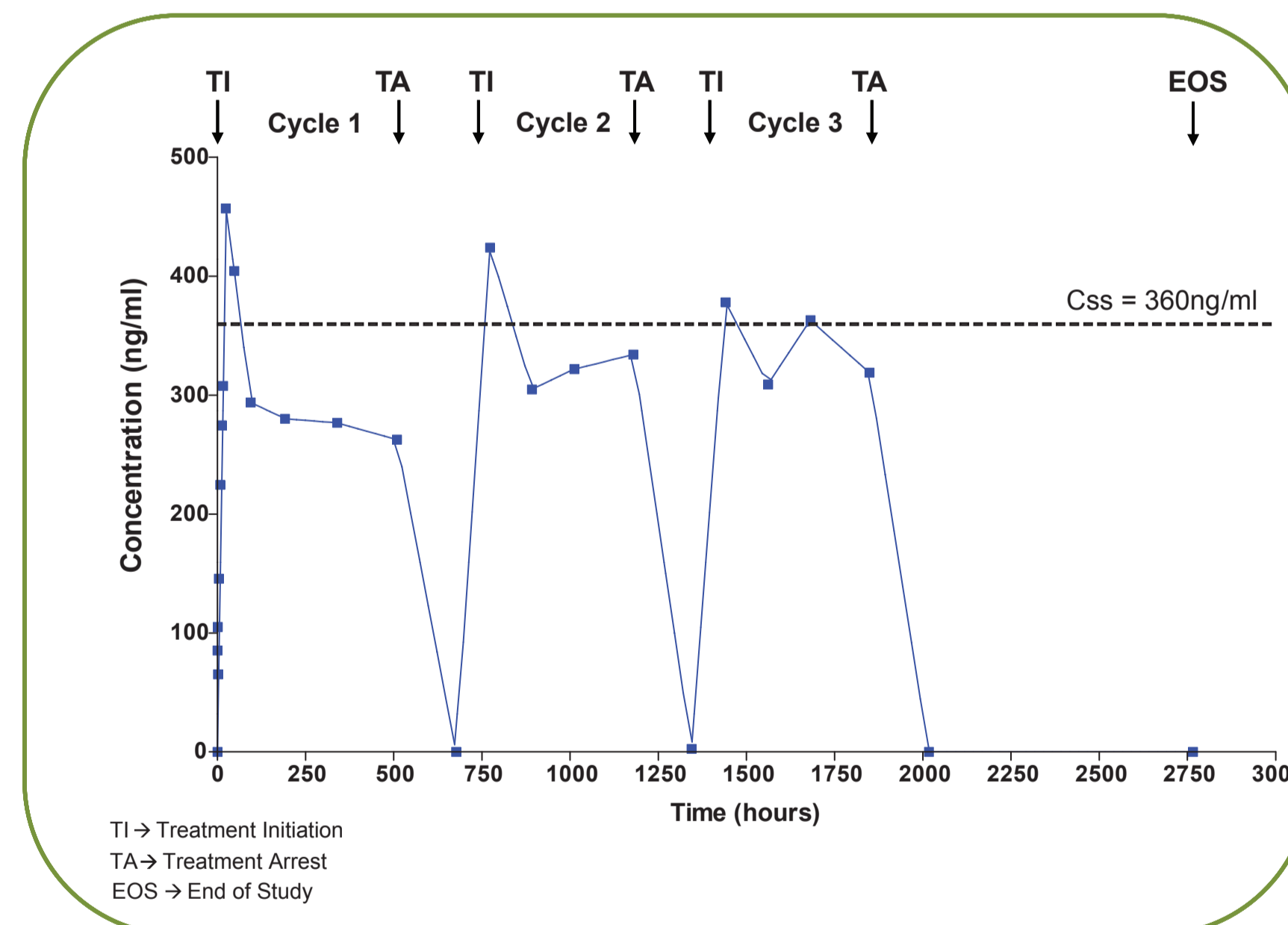
PET scans were conducted during the study and results correlated with CT-scan showing disease progression (increase in lesion size or new lesions).

Pharmacokinetics:

After intravenous administration of TLN-232 at a rate 0.48 mg/kg/day for 21 days, CL averaged 63.5 ± 8.14 mL/h/kg, Vz averaged 541 ± 187 mL/kg, and t_{1/2} averaged 6.36 ± 2.14 h.

Overall, the steady-state concentration and AUC for a 21-day infusion averaged 320 ± 39.7 ng/mL and 161 ± 20.0 h×ng/mL, respectively.

Figure 1: Typical PK Profile (Patient #008)



M2PK:

M2PK has been investigated previously as a potential marker for renal cell carcinoma. In this study we assessed its potential role as a marker for tumor response.

M2PK status of available tumor biopsy samples was analyzed using IHC staining and serum M2PK levels were quantified using a commercial ELISA assay (ScheBo® Tumor M2-PK EDTA plasma test; ScheBo Biotech AG, Germany).

This exploratory analysis demonstrated high variability in M2PK levels among patients. Out of the 6 patients that were evaluable for tissue M2PK, 4 (patients #001, **#004**, **#005**, and #007) showed moderate to strong staining, 1 weak staining (patient #008) and 1 heterogeneous staining (patient #010). M2PK levels in serum were also highly variable, with a wide range from 20 U/ml up to 310 U/ml. The test kit used allows the quantification of tumor M2PK within the range of 5 to 100 U/ml EDTA-plasma. Out of the 10 patients, 3 (patients #002, #008 and #010) had values above 100 U/ml. M2PK blood levels in patients with SD after 3 cycles remained stable (patient **#005**) or moderately increased (patient **#004**). There was no apparent correlation between tissue and blood levels. Due to the limited number of patients and the exploratory nature of the analysis, no definitive conclusions can be drawn.

Safety Results:

TLN-232 was generally safe and well tolerated. Two (2) patients discontinued before completing 3 cycles due to AEs unrelated to study medication; an additional patient discontinued due to "withdrawal of consent". The most frequent adverse events (>20%) are summarized in Table 2:

**Table 2
Most Frequently Reported Adverse Events: Incidence & Episodes**

Preferred Term (Body System)	Number of Patients N = 10	Number of Episodes
Asthenia (General disorders and administration site conditions)	8	20
Nausea (Gastrointestinal disorders)	7	25
Pyrexia (General disorders and administration site conditions)	7	20
Vomiting (Gastrointestinal disorders)	6	17
Hypercalcaemia (Metabolism and nutrition disorders)	5	17
Constipation (Gastrointestinal disorders)	5	7
Anorexia (Metabolism and nutrition disorders)	4	13
Weight decreased (Investigations)	4	8
Abdominal pain (Gastrointestinal disorders)	4	7
Anaemia (Blood and lymphatic system disorders)	4	7
Peripheral Neuropathy (Nervous system disorders)	4	7
Mucosal inflammation (General disorders and administration site cond	4	5
Oedema peripheral (General disorders and administration site condition	4	5
Diarrhoea (Gastrointestinal disorders)	4	4
Hyperglycaemia (Metabolism and nutrition disorders)	3	7
Hyponatraemia (Metabolism and nutrition disorders)	3	7
Flank pain (Musculoskeletal and connective tissue disorders)	3	5
Cough (Respiratory, thoracic and mediastinal disorders)	3	4
Pain (General disorders and administration site conditions)	3	4
Vertigo (Ear and labyrinth disorders)	3	3
Central line infection (Infections and infestations)	3	3
Nasopharyngitis (Infections and infestations)	3	3
Urinary retention (Renal and urinary disorders)	3	3

Related AEs (possible, probable, definite, per investigator's assessment) reported for 2 or more patients were: nausea, peripheral neuropathy, hyperglycaemia, mucosal inflammation, asthenia, diarrhea, pallor, and vomiting. The four cases of peripheral neuropathy were all mild to moderate.

A total of 24 Serious Adverse Events (SAEs) were reported during the study. All were "Unrelated" or "Unlikely related" to the study treatment, except one SAE.

Patient #004 had 1 SAE possibly related to study treatment: GI haemorrhage. This patient was known to have a prior history of epigastralgia and developed on study a GI haemorrhage due to a bulb ulcer. The SAE resolved without sequelae and the patient continued in the study. There was no action taken with the study drug. No death was reported between the first-dose and the 30-day follow-up period after last dose.

CONCLUSIONS

- Signs of anti-tumor activity were detected in advanced renal cell carcinoma patients after 3 cycles of TLN-232 treatment based on the 2 stable diseases**
- The pharmacokinetic profile of TLN-232 was well described by a one-compartment model, showing that the product is rapidly eliminated following infusion**
- TLN-232 administered in continuous infusion over 21 days at a dose of 0.48 mg/kg/day was found to be generally safe and well tolerated in this study**
- Safety and PK results from this pilot study validated the feasibility of a 28-day continuous intravenous infusion regimen for a recently initiated Phase II study in metastatic melanoma**

* Formerly CAP-232