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## BACKGROUND

TLN-4601 is a structurally novel farnesylated dibenzodiazepinone discovered through DECIPHER<sup>®</sup>, a proprietary drug discovery platform. The compound was shown to have broad cytotoxic activity (low  $\mu\text{mol/l}$ ) when tested in the NCI 60 tumor cell line panel and has shown *in-vivo* antitumor activity in several xenograft models. Its inhibition of the MEK/ERK pathway is not mediated by direct kinase inhibition, but appears to be at the level of Raf-1. Interestingly, TLN-4601 induces Raf-1 proteasomal degradation. One hypothesis is that TLN-4601 may inhibit the Ras mitogen-activated protein kinase signaling (Ras/MAPK) pathway by depleting the Raf-1 protein. (ref 1)

A Phase I study of TLN-4601 in patients with solid tumors demonstrated preliminary evidence of anti-tumor activity with 4 of 7 evaluable patients (2 colorectal ca, 1 duodenal ca and 1 ovarian ca) achieving disease stabilization for 6 cycles. (ref 2) TLN-4601 was generally safe and well tolerated. Adverse events potentially related to study drug were nonspecific, not correlated to dose levels, and common in this type of advanced refractory cancers. Pharmacokinetic data revealed that estimated therapeutic TLN-4601 plasma concentrations, based on preclinical animal models, were reached at higher doses and were rapidly eliminated following infusion. Data supported a three-week dosing regimen with CIV administration of drug at 480 mg/m<sup>2</sup>/day over 14 days followed by one week off in this population. (Figure 1)

Figure 1. PK data from Phase I study (ref 3)

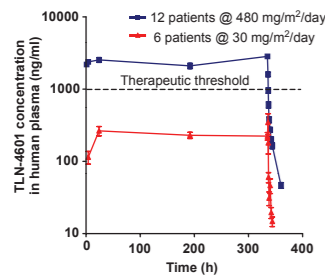
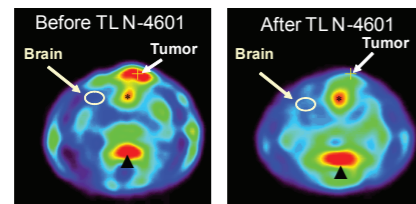


Figure 2. *In vivo* PET scan image (11C-(R)-PK11195) in orthotopic rat model (ref 4)



A Phase II trial of TLN-4601 in patients with glioblastoma multiforme (GBM) at first progression was undertaken because the Ras/MAPK signaling pathway is frequently activated in this cancer type, and preclinical animal data have demonstrated that TLN-4601 crosses the blood brain barrier and accumulates preferentially in implanted brain tumors (Figure 2).

## STUDY DESIGN

A single-arm monotherapy Phase II study was conducted in 8 centers across Canada and the USA. TLN-4601 was administered as a continuous intravenous therapy at a dose of 480 mg/m<sup>2</sup>/day using a central venous line (portacath like system) following a three-week cycle scheme (2 weeks on therapy followed by one week off treatment). Patients were followed for safety and efficacy for a maximum of one year post-initiation of treatment. Safety was assessed according to CTCAE version 3.0. MacDonald criteria were used for tumor response assessments. MRIs were performed at the end of every second cycle until progression. Patients were considered evaluable if they had at least 14 days of continuous drug infusion and at least one post-baseline MRI evaluation. Pharmacokinetic analyses were conducted using a Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) method. Samples were also analyzed for biomarkers, Raf-1 and pERK, using immunostaining and flow cytometry (ref 5).

### References:

- Bouffaiet et al. TLN-4601, A Novel Anticancer Agent, Inhibits Ras Signaling Post Ras Prenylation and before MEK Activation. *Anticancer Drugs*. 2010; 21(5):543-52
- Kavan P. et al, A Phase I study of ECO-4601, a Novel Bifunctional Targeting Agent. *Gastrointestinal Cancers Symposium Science and Multidisciplinary Management of GI Malignancies*, Orlando, 2008
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- Gourdeau et al. Targeted delivery of ECO-4601 to Brain Tumors in an Orthotopic Model. *3rd Modern Drug Discovery & Development Summit* San Francisco, 2007
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## RESULTS

### Safety

A total of 179 adverse events were observed with the following reported relationship to TLN-4601: 1 as definitely related (headache), 1 as probably related (fatigue) and 11 as possibly related (6 of them for fatigue/asthenia).

Table 2. Adverse Events reported ( $\geq 10\%$ )

Preferred Term	No. of Patients with AEs		Preferred Term	No. of Patients with AEs	
	Total = 20	No. of AEs Total = 179		Total = 20	No. of AEs Total = 179
	N (%)	N		N (%)	N
Asthenia/fatigue	12 (60)	5	Diabetes mellitus	2 (10)	2
Headache	6 (30)	8	Hypokalemia	2 (10)	2
Catheter site erythema	5 (25)	5	Arthralgia	2 (10)	2
Muscular weakness	5 (25)	6	Aphasia	2 (10)	2
Anxiety	4 (20)	5	Ataxia	2 (10)	2
Convulsions	4 (20)	4	Facial palsy	2 (10)	2
Hemiparesis	3 (15)	3	Hyperreflexia	2 (10)	2
Catheter infection/sepsis	3 (15)	3	Partial seizures	2 (10)	2
Oral candidiasis	3 (15)	3	Peripheral neuropathy	2 (10)	2
Tachycardia	3 (15)	3	Somnolence	2 (10)	2
Constipation	3 (15)	3	Agitation	2 (10)	2
Nausea	3 (15)	3	Confusional state	2 (10)	4
Diarrhea	2 (10)	4	Insomnia	2 (10)	2
Ear discomfort	2 (10)	2	Dyspnoea	2 (10)	2
Blurred vision	2 (10)	2	Pulmonary embolism	2 (10)	2
Pyrexia	2 (10)	2	Erythema	2 (10)	2
Oedema peripheral	2 (10)	2			

Table 1. Patient Demographics

Variable	Parameter	All
Race	White	19 (95%)
	Black (African Heritage )	1 (5%)
Body Weight (kg)	Mean	81 Kg
ECOG	Mean	0.7
Previous GBM history	Surgery (debulking)	16 (80%)
	Radiotherapy	20 (100%)
Total Dose (Gy)		60 (except for 1 pt)
	Fist-line therapy	
	1) Temozolomide	20 (100%)
	2) mTor inhibitor	1 (5%)

Pharmacokinetic data were compatible with typical parameters of continuous intravenous administration with sustained drug levels of approximately 1800 ng/mL, corresponding to 3.9  $\mu\text{mol/L}$ . All patients demonstrated signs of drug exposure at targeted levels as per *in vivo* models (2-5  $\mu\text{mol/L}$ ), and with similar pharmacodynamics as observed in the Phase I study. (ref 3)

A total of 9 Serious Adverse Events (SAEs) were reported in 4 patients, 1 being deemed potentially drug-related (DIC).

Table 3. Serious Adverse Event listing

Patient ID	Event Term	Grade	Action Taken	AE Outcome	Reporter Causality
01-002	Confusion	3	Drug withdrawn	Not Recovered / Not Resolved	Unrelated
03-001	Diabetic ketoacidosis	4	Drug withdrawn	Recovered / Resolved with sequelae	Unrelated
03-001	Disseminated Intravascular Coagulation	2	Drug withdrawn	Recovered / Resolved	Possible
03-001	Pulmonary Embolism	4	Drug withdrawn	Not Recovered / Not Resolved	Unlikely
03-001	Septicemia secondary to catheter infection	3	Drug withdrawn	Not Recovered / Not Resolved	Unrelated
03-003	Staphylococcus Epidermidis Infection	2	Dose not changed	Recovered / Resolved	Unrelated
03-003	Septicemia secondary to catheter infection	3	Drug withdrawn	Recovered / Resolved	Unrelated
03-003	Pulmonary embolism	4	Not applicable	Recovering / Resolving	Unrelated
07-003	Septicemia secondary to catheter infection	3	Drug withdrawn	Not Recovered / Not Resolved	Unrelated

### Efficacy

Of the 20 patients who received any amount of study drug, a total of 17 patients were evaluable (3 patients discontinued treatment before 14-days of treatment: 2 due to AEs, and 1 due to clinical progression).

All 17 evaluable patients progressed relatively rapidly. Only 3 were still stable after 2 cycles (6 weeks), but progressed after 4 cycles (3 months).

Figure 3. Patients' response to therapy

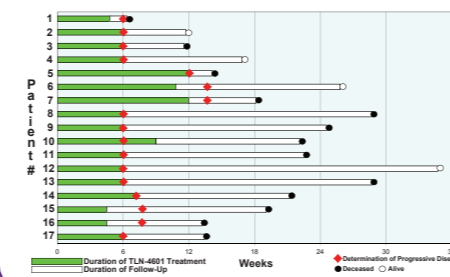
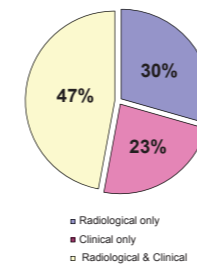


Figure 4. Type of progressive disease (MacDonald)



### Biomarkers

Results from the Raf-1 analysis demonstrated that the majority of patient samples (75%) had no substantial fluctuations of Raf-1 levels. Among the three patients that had stable disease for 2 months, two had decreased Raf-1 levels (nadirs: 47% reduction at 6 weeks when patient 03/03 was declared stable; 27% reduction at 12 weeks when patient 03/04 was declared in progression).

Results for pERK indicated that the majority of samples showed decreased levels on treatment (50% for granulocytes/monocytes and 75% for lymphocytes sub-populations). pERK levels also decreased in the three patients that had stable disease for 6 weeks, although it was only transient, with a nadir before they reached the 6-week time point.

## CONCLUSIONS

Lack of clinical efficacy signal of TLN-4601 monotherapy in the 20 GBM patients enrolled led to the discontinuation of this Phase II study at the planned interim analysis. Only 3 patients had slightly delayed progression of a few weeks which did not support further patient exposure to drug. Although the drug seemed relatively well tolerated, adverse events related to central line usage were observed, including clotting and severe infections. The limited sample size and data variability make the biomarker results difficult to interpret. Re-evaluation of the potential of TLN-4601 in other indications or using different regimens is warranted.