

## **DRUG DISCOVERY RATIONALE**

TLK58747 is a tetrachloro-substituted sulfonylethyl phosphorodiamidate prodrug capable of releasing a reactive alkylating entity under physiological conditions. The alkylating portion, tetrakis(2-chloroethyl)-phosphorodiamidic acid, is a symmetrical moiety that reacts with DNA and other biomolecules to induce apoptosis. The high chlorine content of this molecule greatly increases its lipophilicity, which may facilitate its transfer across cellular membranes. Due to its chemical structure, TLK58747 does not produce acrolein when metabolized and consequently may not exhibit the urothelial toxicity of CPA and other related alkylating drugs.

Since their discovery and subsequent introduction into clinical practice, alkylating agents have become a mainstay of cancer treatment. Agents such as chlorambucil, cyclophosphamide (CPA), ifosfamide and BCNU (carmustine) are used to treat a variety of cancers including leukemias, lymphomas and brain tumors, either alone or in combination with other anticancer drugs. New agents with superior safety profiles and novel mechanistic features, such as TLK58747, will be an important addition to these regimens, or may replace current chemotherapeutics due to the occurrence of drug resistance to these other agents.

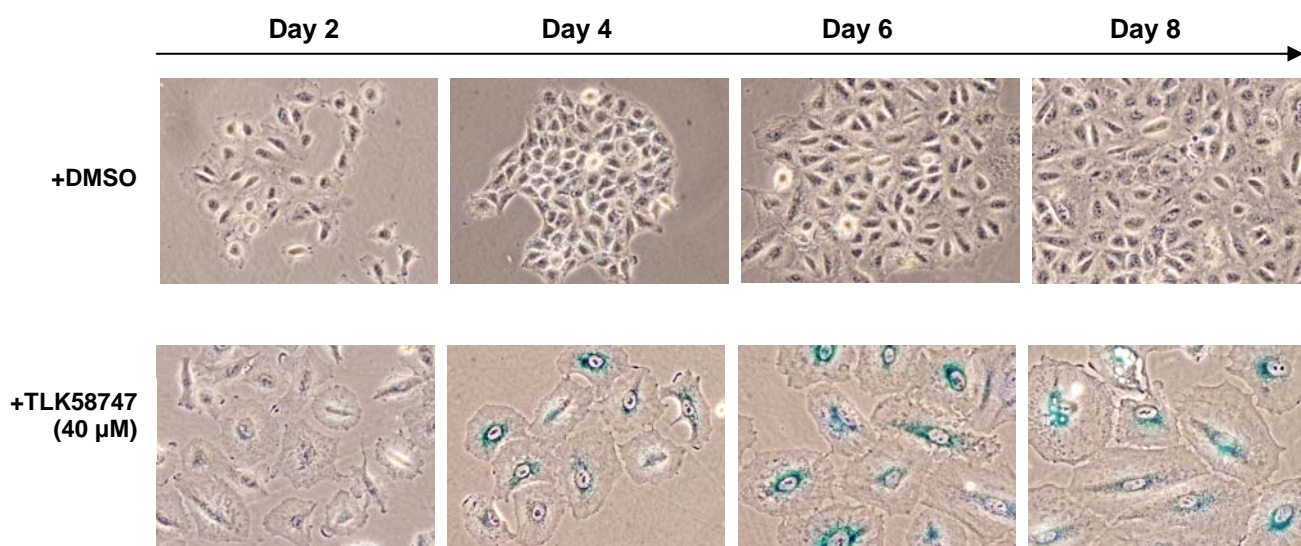
## **ACTIVITY PROFILE**

### ***In vitro* Activity**

TLK58747 inhibited the growth of multiple established human cancer cell lines *in vitro*, including those derived from breast, lung, colorectal, ovarian, pancreatic, and prostate carcinomas as well as glioma and promyelocytic leukemia. Importantly, TLK58747 retained its effectiveness against P388ADR, a multi-drug-resistant leukemia cell line, and against derivatives of the human ovarian cancer cell line OVCAR3 that were selected for resistance to carboplatin or paclitaxel. Less than 2-fold resistance was detected with TLK58747 in a derivative of the OVCAR3 cell line that was approximately 9-fold resistant to carboplatin. Similarly, only a low level (approximately 3-fold) of cross-resistance was observed in cancer cell lines that were more than 30-fold resistant to adriamycin or paclitaxel.

Treatment of human tumor cell lines with TLK58747 can lead either to senescence or apoptosis, which may correlate with the cells' p53 status. TLK58747 treatment of these cells activated the DNA damage response pathway, as indicated by increased levels of the histone protein  $\gamma$ H2AX, phospho-checkpoint kinase 1 (p-chk1), and phospho-checkpoint kinase 2 (p-cdk-2). Increased phosphorylation on the negative regulatory site of cell division control protein 2 (cdc2), the key regulator of G2/M transition, was also observed. In addition, TLK58747 induced pronounced G2/M cell cycle arrest in multiple human solid tumor cell lines. TLK58747 treatment produced dose- and time-dependent premature senescence, as characterized by enlarged flat cells that stain positive for senescence-associated  $\beta$ -galactosidase, in A549 cells which are p53- positive (see figure below), while apoptosis was observed in OVCAR3 cells, which are p53- negative.

**Time Course of TLK58747 in A549 Human Lung Cancer Cells**

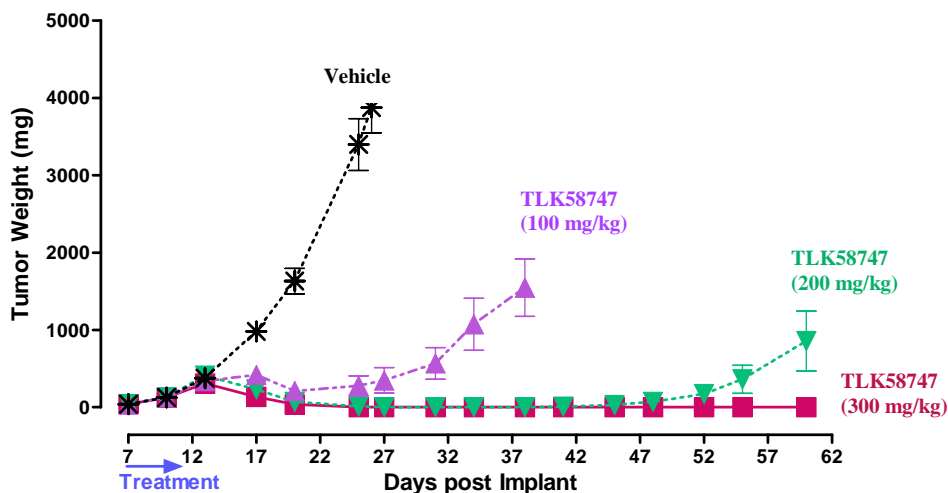


***In vivo* Activity**

TLK58747 demonstrated significant activity when given orally and parenterally against a wide variety of human tumor xenografts. It strongly inhibited the growth of MX-1 human breast carcinoma xenografts, resulting in complete tumor regression in all animals (10/10) treated orally at 300 mg/kg (q.d.x 5) and in 4 of 10 treated orally at 200 mg/kg (q.d. x 5) (see figure below). TLK58747 also showed significant activity in other human tumor xenograft models, including colorectal and prostate cancer, as well as in glioma and promyelocytic leukemia. Elevated brain

concentrations of TLK58747 were observed upon IV administration, indicating the passage of the compound across the blood-brain barrier. Potent anti-tumor activity was also observed in the xenograft model of human pancreatic cancer, a tumor type that is known to be insensitive or resistant to standard alkylating agents.

**Oral Activity of TLK58747 in MX-1 Human Breast Cancer Xenograft**



TLK58747 appears to possess a safety profile that is superior to conventional alkylating agents such as CPA. TLK58747 caused significantly less hematological toxicity than CPA, and unlike CPA, TLK58747 had minimal effects on red blood cells and platelets. Moreover, the reduction of neutrophil counts with TLK58747 was less pronounced and showed faster recovery than with CPA. At the efficacious doses tested, TLK58747 caused no mortality or significant body weight loss.

## CONTACT INFORMATION

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