I. *In vitro* effects of prasugrel metabolites on proliferation of human tumor cell lines:

Study no.: CCGSO2
Conducting laboratory: Eli Lilly & Co, Indianapolis, IN
Report date: Jan 23, 2009
Drug: R-138727 and R-106583 (Prasugrel metabolites)

Methods:

Strains/cell line: Human tumor cell lines NCI-H460 (non-small cell lung carcinoma), HCT-116 (colon carcinoma), and PC-3 (prostate carcinoma).

Doses used in study: 70 and 700 ng/ml (approximately 1x and 10x, human plasma Cmax levels receiving 10 mg prasugrel).

Negative control: Serum-free media

Positive control: Enhanced proliferation to 10% FBS in serum-starved cells for 24 or 48 hours.

Incubation and sampling times: Cells were serum-starved for 24 or 48 hours followed by treatment for 24 or 48 hours with the metabolites. Following overnight incubation, the 10% FBS-containing media was removed and serum-free media (or media containing 10% FBS) was added back for a 24 or 48 hour starvation period. Serum-starved cells were also treated by adding back 10% FBS for 24 or 48 hours as a positive control for enhanced cellular proliferation. PC-3 cells after 24 hrs starvation and NCI-H460 cells after 48 hrs starvation were not evaluated due to the inability to establish a suitable signal window. Cell proliferation was analyzed by the conversion of tetrazolium salt (WST-1 reagent) to soluble formazan via the glycolytic production of NAD(P)H in viable cells, and the amount of dye formed was quantitated by spectrophotometer. The experiments were repeated 3 separate times and contained 6 replicates per data point.

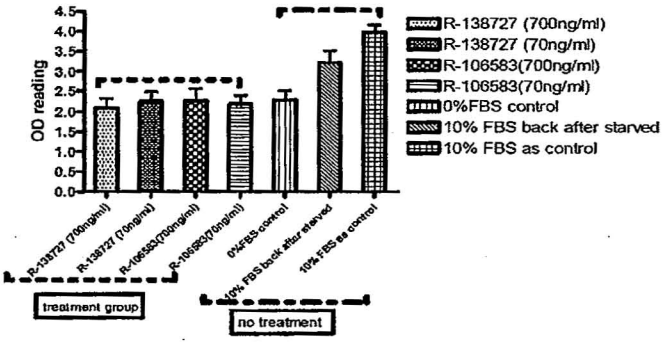
Results:

Treatment of NCI-H460, HCT-116 and PC-3 tumor cells with either the high or low doses of prasugrel metabolites did not enhance cellular proliferation relative to the group that received media containing 10% FBS following serum starvation (Figure 1: Panels A, B and C). The FBS-starved cells responded to a tumor progression signal upon readdition of 10% FBS (Figure 1).

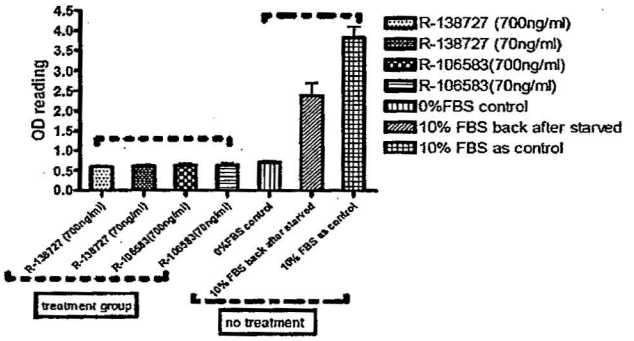
Summary of findings:

The data showed that exposure of serum-starved human tumor cell lines *in vitro* to prasugrel metabolites did not increase cell proliferation relative to starved cells stimulated to proliferate by addition of 10% FBS. The active and inactive prasugrel metabolites (R-138727 and R-106583) did not augment cellular proliferation in the 3 human tumor cell lines at concentrations that approximated 10x human plasma Cmax levels.

Panel A. NCI-H460 human lung carcinoma cells,
24 hrs starvation and 48 hrs treatment at 0% FBS media



Panel B. HCT-116 human colon carcinoma cells,
48 hrs starvation and 48 hrs treatment at 0% FBS media



Panel C. PC3 human prostate carcinoma cells,
48 hrs starvation and 48 hours treatment at 0% FBS media

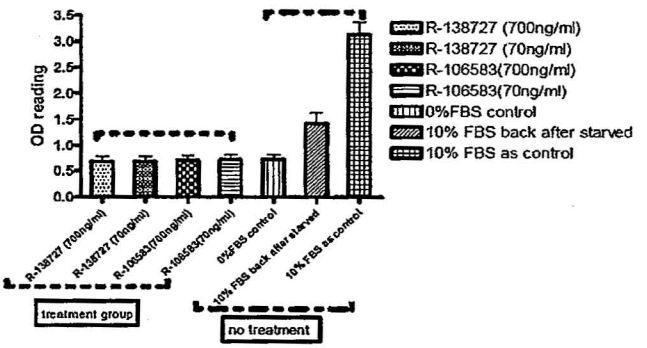


Figure 1. *Panels A, B and C:* **Effect of prasugrel metabolites on the proliferation of NCI-H460, HCT116 and PC3 cells *in vitro*.** Cells were treated with prasugrel metabolites (70 and 700 ng/ml) for 24 or 48 hours. Positive control cells received 10% FBS media for 24 or 48 hours post starvation. Negative control cells were maintained in serum-free media. One group of cells was maintained in 10% FBS media throughout the treatment period as an indicator of maximum proliferation in the absence of starvation. The positive control cells displayed a significant enhancement of cellular proliferation relative to negative control cells, while there was no effect on cell growth by prasugrel metabolites.

II. *In vivo* effects of prasugrel on growth rates of human tumor xenografts in nude mice

Study no.: CCGSO3
Conducting laboratory: Eli Lilly & Co, Indianapolis, IN
Report date: Jan 23, 2009
Drug: Prasugrel.HCl

Strains/species: Nude mice implanted subcutaneously with human tumor xenografts of NCI-H460 non-small cell lung carcinoma, HCT-116 colon carcinoma, and PC-3 prostate carcinoma cell lines (n=10/group).

Doses used in study: 1 and 10 mg/kg/day as suspension by oral gavage.

Vehicle: Tragacanth gum/saline 0.5%

Basis of dose selection: A separate 14-days exposure study indicated that the plasma metabolite levels (Cmax and AUC) in the NCI-H460 human tumor-bearing nude mice at a dose of 1 or 10 mg/kg/d prasugrel were similar to or 20- to 46-fold higher, respectively, than the human exposure levels (Table 1).

Table 1. Exposure levels of R-138727 and R-106583 following multiple oral doses of Prasugrel for 2 weeks in NCI-H460 tumor bearing mice (n=36/group)

Dose[a]	Study Day	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (ng-hr/mL)
		R-138727		
1 mg/kg	1	165.4[b]	1.0	263.5[b]
	14	97.6	0.5	135.5
10 mg/kg	1	1610.7[c]	0.5	2299.8[c]
	14	1516.6	0.5	2237.1
		R-106583		
1 mg/kg	1	97.8	0.5	299.8
	14	112.6	0.5	253.8
10 mg/kg	1	2906.0	0.5	6090.7
	14	2746.6	0.5	6641.3

Data for Humans (TAAV study): Cmax (ng/ml) AUC (ng.hr/ml)
R-138727 70 67
R-106583 63 288

Methods:

Tumors were allowed to grow to approximately to 100 mm^3 prior to initiation of treatment. The mice containing the HCT-116 colon and PC-3 prostate human tumor xenografts received 26 or 28 daily oral doses of prasugrel, respectively, while the mice bearing the NCI-H460 human lung tumor xenografts received 13 daily doses due to the rapid rate of tumor growth including that in the vehicle control which necessitated an early termination. Tumors were measured twice weekly by calipers and tumor volume (mm^3) was estimated from the formula $l \times w^2 \times 0.53$, where l is the larger and w is the smaller of perpendicular diameters. At the end, tumors were excised and weighed in a blinded fashion to determine the accuracy of the estimated tumor volume to actual tumor weights at study termination.

Results:

All treatments were well-tolerated as indicated by minimal weight loss, and no deaths occurred in any group. There was no significant enhancement of tumor growth by prasugrel (1 and 10 mg/kg) in three nude mice models implanted with human tumor derived from lung, colon and prostate (Figure 2).

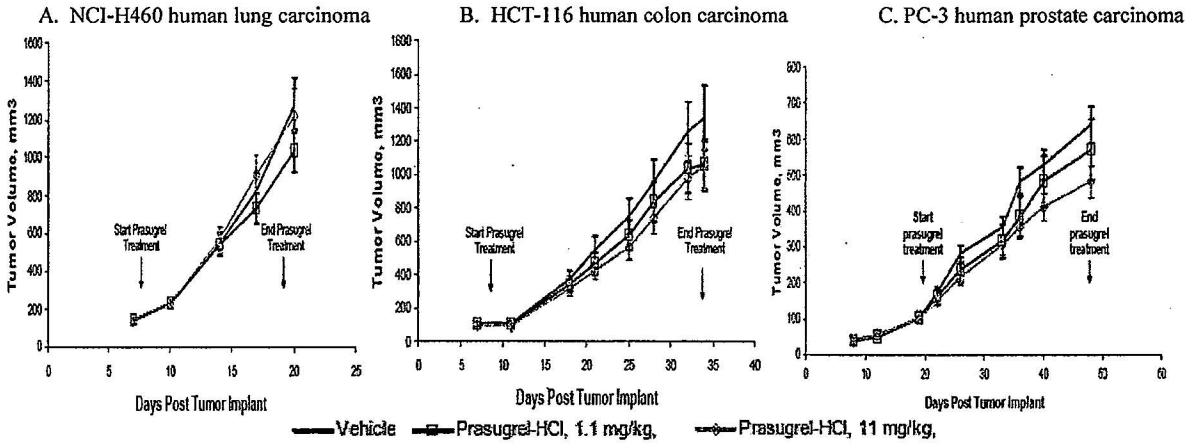


Figure 2: **Effect of prasugrel.HCl on the proliferation of PC-3 human prostate, HCT-116 human colon and NCI human lung carcinoma xenografts.** Nude mice were implanted subcutaneously with tumor cells. Prasugrel or vehicle was administered by oral gavage daily. No enhanced tumor growth relative to vehicle control was observed for either dose of prasugrel (n=10/group).

Summary of findings:

Treatment of nude mice bearing 3 different human tumor xenografts with prasugrel at doses up to an order of magnitude greater than the human plasma levels of active and inactive metabolites had no discernable effect on tumor growth relative to vehicle control.

Conclusions

- *In vitro* exposure of serum-starved human tumor cell lines to prasugrel metabolites, the active (R-138727) and inactive (R-106583), did not increase cell proliferation relative to starved cells stimulated to proliferate by addition of 10% FBS to human tumor cell lines NCI-H460 (non-small cell lung carcinoma), HCT-116 (colon carcinoma), and PC-3 (prostate carcinoma).

- *In vivo* tumor growth rates were not enhanced by treatment with prasugrel (1 and 10 mg/kg for 13-28 days) in tumor-bearing nude mice based on rate of calculated tumor volume at autopsy. Adequate exposure to the active (R-138727), and inactive (R-106583) metabolites were obtained in the NCI-H460 human tumor-bearing nude mice (about 20-fold higher than human exposure).

- In conclusion, the *in vitro* and *in vivo* tumor progression studies suggest that prasugrel is unlikely to exhibit tumor growth enhancement activity.

Study Limitations:

- In the *in vitro* studies, the ability of starved cells to respond to a chemical entity, e.g., lysophosphatidic acid, was not tested.
- The *in vitro* studies should have been tested at several concentrations of FBS (e.g., 0.5, 1, 2.5, 5, 10%), instead of 0%FBS.
- In the *in vitro* studies, serum starvation (i.e., lack of growth factors) reduces or eliminates the ability of cells to proliferate. Without a true positive control (i.e., a drug that is known to enhance tumor cell proliferation), it can not be determined whether any drug would have produced an increase in cell growth.
- In the *in vivo* studies, no positive controls were used to demonstrate that these tumor cells have the capability to grow faster. The tumor cell lines used appeared to have rapid doubling times already. It may not be possible to increase such rapid growth any further with any agent. The issue for human risk assessment is whether the growth of slow growing tumors can be increased by prasugrel (i.e., tumor promotion).
- Histopathological examination were not performed on tumor tissues excised from the nude mice implanted with human tumor cell lines.

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