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The AXL inhibitor, TP-0903, reverses EMT and shows activity in non-small cell lung cancer preclinical models

Abstract 160

Ray M. Joe[1], Yuta Matsumura[1], Adam Siddiqui[1], Jason M. Foulks[1], Muhammad Beg[2], Jonathan Thompson[3], Noboru Yamamoto[4], Alexander Spira[5], John Sarantopoulos[6], Jason Melear[7], Yanyan Lou[8], Claudia Lebedinsky[9], Jian Li[9], Akinobu Watanabe[9], and Steven L. Warner[1]

1) Sumitomo Pharma Oncology, Inc., Lehi, UT, USA 2) University of Texas – Southwestern, Dallas, TX, USA, 3) Medical College of Wisconsin, Milwaukee, WI, USA, 4) National Cancer Center Hospital, Tokyo, Japan 5) Virginia Cancer Specialists, Fairfax, VA, USA, 6) Institute for Drug Development, Mays Cancer Center at University of Texas Health San Antonio , San Antonio, TX, USA, 7) Texas Oncology, Austin, TX, USA, 8) Mayo Clinic, Jacksonville, FL, USA, 9) Sumitomo Pharma Oncology, Inc., Cambridge, MA

I. Abstract

Background:

Adenocarinomas frequently adopt mesenchymal properties to become metastatic, aggressive and drug-resistant. Non-small cell lung cancer (NSCLC), a leading cause of cancer related deaths, is a well understood cancer type to utilize an epithelial-to-mesenchymal transition (EMT) to acquire drug resistance in preclinical models. This is particularly applicable in resistance to EGFR inhibitors (e.g. osimertinib) in EGFR+ NSCLC, where resistance has been linked to EMT and increased AXL expression, a known driver of the mesenchymal phenotype. TP-0903 is an investigational potent multi-targeted kinase inhibitor shown to reverse the mesenchymal phenotype through AXL inhibition and other mechanisms in preclinical models. TP-0903 and osimertinib have shown synergistic activity in the EGFR+ H1650 xenograft model. TP-0903 is currently being evaluated in combination with EGFR inhibitors in EGFR+ NSCLC patients (NCT02729298).

Materials and Methods:

To investigate in vitro cytotoxicity and EMT markers in mutant EGFR NSCLC cell lines, H1650 and H1675, CellTiter Glo assay and western blots were performed, respectively.
In a Phase I clinical trial, patients with EGFR+ NSCLC and recent progression following a best response of SD, PR, or CR per RECIST v1.1 on ≤2 lines of oral TKIs were treated with TP-0903 once daily for 21 out of 28 days plus (add-on) EGFR TKI to evaluate safety and preliminary antitumor activity.

Results:

In an in vitro cytotoxicity assay, TP-0903 resulted in IC50 values of 193.3 nM and 40.15 nM in H1650 and H1975 cell lines, respectively. TP-0903 was observed to reduce SNAIL expression in H1650 and H1975. Data showed TP-0903 reduced SLUG expression and the combination treatment synergized to reduce SNAIL and SLUG expression to a further extent in H1975. Additional cell viability and EMT markers will be evaluated in NSCLC cell lines and patient derived samples.

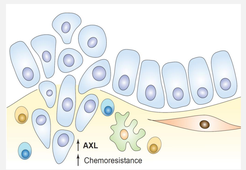
As of April 22, 2022, 22 patients (median age 64 y, 64% women, 59% white and 32% Asian, median 2 [range, 1-7] prior lines, 18 had osimertinib as last line) were treated with TP-0903 (3 at 37 mg flat dose, or 19 at 50 mg flat dose) as add-on to EGFR inhibitor. The overall response rate (ORR) by investigator is 1 (4.5%) partial response (PR) and 13 (59%) stable disease (SDs), with 6 SDs for >6 months. Disease control rate (DCR) is 64%. One pt with EGFR exon 19 deletion who progressed on osimertinib has an ongoing PR for 3+ years. The most common TEAEs were nausea, vomiting and diarrhea, majority were mild and manageable with supportive care.

Conclusions:

TP-0903 was observed to be active and suppressed EMT marker expression in mutant EGFR cell lines. TP-0903 added to osimertinib in unselected resistant EGFR+ NSCLC patients showed signals of activity that warrants further investigation; the safety profile was manageable.

II. Background

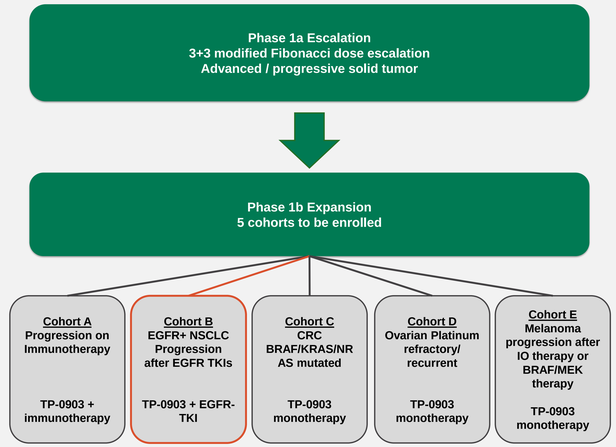
Figure 1: Axl as a potential target to overcome drug resistance

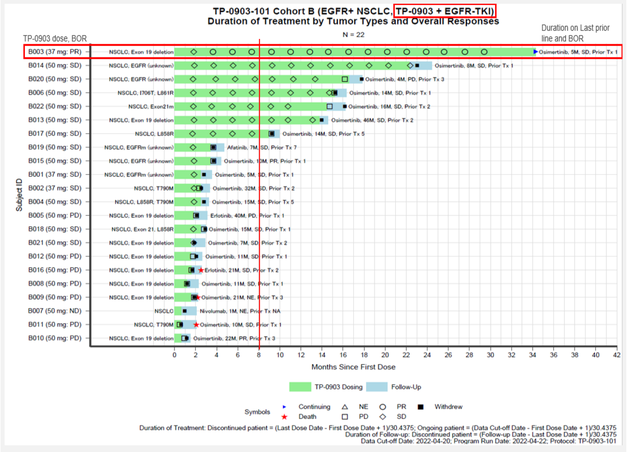


- AXL is the prototypical member of the TAM family (including TYRO3 and MER) of receptor tyrosine kinases.
- AXL promotes the epithelial-to-mesenchymal transition (EMT).
- AXL overexpression has been reported in numerous tumors, especially following therapeutic treatment.
- TP-0903, an investigational potent Axl inhibitor, and osimertinib has been shown to have anti-tumor activity in NCI-H1650 and NCI-1975 xenograft models (in house data, Mangelson, R., et al. AACR 2019)
- Initial clinical investigation of TP-0903 and osimertinib combination therapy has been completed, further studies warranted.

III. Results

Figure 2: In a phase 1 escalation study, TP-0903 and EGFR TKIs showed clinical responses in NSCLC patients





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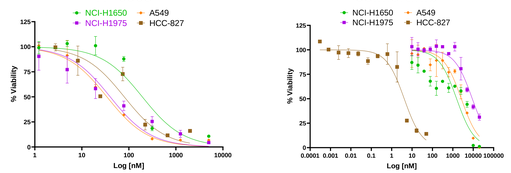
- The overall response rate (ORR) by investigator is 1 (4.5%) partial response (PR) and 13 (59%) stable disease (SDs). Disease control rate (DCR) is 64%.
- One patient with EGFR exon 19 deletion who progressed on osimertinib has an ongoing PR after TP-0903 was added to osimertinib for 3+ years.
- Seven patients (1 PR, 6 SD) remained on study treatment >8 months after progression on prior TKIs.
- The most common TEAEs were nausea, vomiting and diarrhea, majority were mild and manageable with supportive care.

Figure 3: A549, NCI-H1975, HCC-827, and NCI-1650 NSCLC cell lines show sensitivity to TP-0903



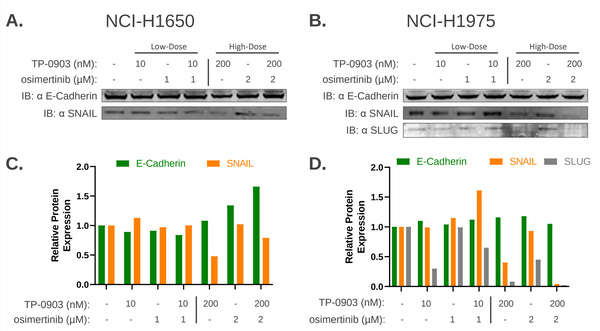
TP-0903 and osimertinib activity in the NCI-H1650, NCI-H1975, A549, and HCC-827 NSCLC cell lines. Cells were incubated in the presence of TP-0903 or osimertinib for 72h, following which cell viability was assessed using the CellTiter-Glo reagent according to manufacturer protocol. IC50s for TP-0903 and osimertinib are shown in figure 4.

Figure 4: Additive combination response is observed between TP-0903 and osimertinib in NSCLC cell lines

Cell line	EGFR Mutation	Axl Expression (Depmap)	TP-0903/osimertinib Synergy (ZIP)	TP-0903 IC50 (nM)	osimertinib IC50 (nM)
A549	WT	5.97	-0.76	34.15	1532.00
NCI-H1975	T790M, L858R	4.77	-3.35	40.15	9005.00
HCC-827	E746_A750del	2.34	-5.47	79.82	4.13
NCI-H1650	E746_A750del	5.51	-7.70	193.30	2601.00

TP-0903 and osimertinib combination synergy study in A549, NCI-H1975, NCI-H1650, and HCC-827 NSCLC cell lines in the presence of TP-0903, osimertinib, or a combination TP-0903 and osimertinib for 72 hours. Drug combination synergy was evaluated by a 12x7 dose-response matrix of osimertinib and TP-0903 in NCI-H1650, NCI-H1975, and A549 cells. In HCC-827 cells, drug combination synergy was evaluated by a 7x12 dose-response matrix of osimertinib and TP-0903. Cell viability was assessed using the CellTiter-Glo reagent according to manufacturer protocol. The dose-response matrix was inputted into the webtool at https://www.synergyfinder.org (see citation) to determine the ZIP synergy score. Antagonistic response ≤ -10, Additive response between -10 to +10, and Synergistic response = ≥+10.

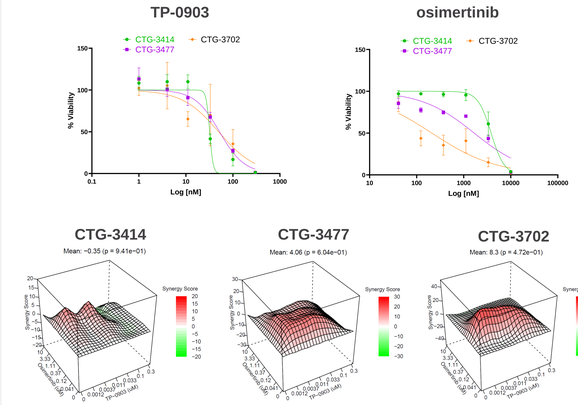
Figure 5: TP-0903 shown to maintain E-Cadherin and reduce SNAIL protein expression in NCI-H1650 and NCI-H1975 NSCLC cell lines. TP-0903 observed to reduce SLUG protein expression in NCI-1975.



EMT marker protein expression in TP-0903, osimertinib, or TP-0903 + osimertinib treated NCI-H1650 and NCI-H1975 cells. (**Fig. A, B**) NCI-H1650 and NCI-H1975 cells were treated with indicated doses of TP-0903, osimertinib, or TP-0903 + osimertinib, for 24 hours, following which cells were harvested, lysed, and protein normalized by BCA. E-Cadherin, SNAIL, and SLUG protein expression was assessed using standard western immunoblotting technique. (**Fig. C, D**) The relative signal intensities from E-Cadherin, SNAIL and SLUG were normalized to the total protein for each lane and further normalized to the dimethyl sulfoxide (DMSO) treated signal.

Figure 6: Osimertinib resistant patient-derived *ex vivo* tumors sensitive to osimertinib and TP-0903 combination therapy

ID	Clinical osimertinib response	EGFR mutation Amino acid change	VAF	MET CNV	mRNA (Log (RPKM+1)) Axl	E-Cadherin	SNAIL	SLUG	Synergy Score (Zip)	IC50, nM TP-0903	osimertinib
CTG-3414	Responded; then progressed	Exon 19 deletion (Glu746_Ala750del)	0.62	11	6.3	0.9	1.5	5.5	-0.35	32.1	3830.0
		T790M	0.39								
CTG-3477	No response	Exon 19 deletion (Leu747_Pro753delinsSer)	0.49	3	1.2	2.3	0.3	0	4.06	51.9	1682.0
		T790M	0.29								
		E865G	0.22								
CTG-3702	No response	No EGFR mutation	-	NA	3.9	7.2	2.5	0.5	8.3	46.0	235.0



TP-0903 and osimertinib combination synergy study on *ex vivo* patient tumors in the presence of TP-0903, osimertinib, or a combination TP-0903 and osimertinib for 5-days. Independent TP-0903, osimertinib or combination activity was evaluated by a 6x6 dose-response matrix of osimertinib and TP-0903 in indicated cells. Cell viability was assessed using the CellTiter-Glo reagent according to manufacturer protocol. IC50s for TP-0903 and osimertinib are shown in the figure 7 table. The dose-response matrix was inputted into the webtool at https://www.synergyfinder.org (see citation) to determine the ZIP synergy score. Antagonistic response ≤ -10, Additive response between -10 to +10, and Synergistic response = ≥+10.

IV. Conclusions

- TP-0903 added to osimertinib in unselected resistant EGFR+ NSCLC patients showed signals of activity that warrants further investigation; the safety profile was manageable.
- TP-0903 observed to reduce SNAIL and maintain E-Cadherin in the EGFR-mutated, osimertinib-resistant NCI-H1650.
- TP-0903 / osimertinib combination reduced SNAIL protein expression in the EGFR-mutated, osimertinib-sensitive NCI-H1975.
- Osimertinib-resistant patient-derived *ex vivo* tumors observed sensitive to TP-0903 and show an additive combination response.

V. Citations

- Mangelson, R., Tyagi,E., Peterson, P., Siddiqui-Jain, A., Whatcott, C.J., Bearss, D.J., and Warner, S.L. "The potent AXL kinase inhibitor, TP-0903, is active in pre-clinical models of EGFR positive non-small cell lung cancer", Abstract 3804, AACR 2019.
- Zheng, S.; Wang, W.; Aldahdooh, J.; Malyutina, A.; Shadbahr, T.; Tanoli Z; Pessia, A.; Jing, T. "SynergyFinder Plus: Toward Better Interpretation and Annotation of Drug Combination Screening Datasets. Genomics", Proteomics & Bioinformatics 2022, in press. doi:10.1016/j.gpb.2022.01.004