# ABSTRACT

Head and neck squamous cell carcinomas (HNSCCs) are aggressive malignancies that develop in the mucosal epithelium of the head and neck region. Despite significant advancement in the development of therapeutic modalities such as immunotherapies, a significant proportion of patients appear with an advanced stage disease. A major reason for it is development of drug resistance that limits the efficacy of the treatment. This indicates the necessity to understand the molecular mechanisms underlying drug resistance and discover additional clinically relevant biomarkers to predict the same in advance.

Toll-like receptors are innate-immune receptors that are activated by conserved molecular signatures derived from pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are molecules derived from microbes and DAMPs are molecules released from host cells due to external triggers like therapy, stress and death. Activation of TLRs by these molecules induces the downstream signaling cascade leading to the production of cytokines and inflammatory mediators. Interleukin-1 receptor-associated kinases (IRAKs), primarily IRAK-1 and IRAK-4, are the active kinases that are critical signal transducers in the TLR signaling pathway. Besides immune cells, TLRs have been found to be expressed on cancer cells and TLR signaling has been shown to contribute in various tumor-promoting attributes like proliferation, survival, migration and inflammation in Breast cancer, Melanoma and Colorectal carcinoma.

In this study, we investigated the pro-oncogenic role of Interleukin-1 receptor-associated kinases (IRAK) mediated Toll-like receptor (TLR) signaling on HNSCC progression along with understanding its impact on chemo-resistance using the laryngeal origin cancer cell line HEp-2. It expressed all TLRs, except TLR 2 and TLR 10 with TLR signaling found to be constitutively ON as reflected by the phosphorylated state of downstream kinases IRAK-1 and IRAK-4. Pharmacological inhibition of TLR signaling pathway through these downstream kinases had a cytostatic effect on HEp-2 cells as reflected by decrease in viable cells count and proliferative potential.

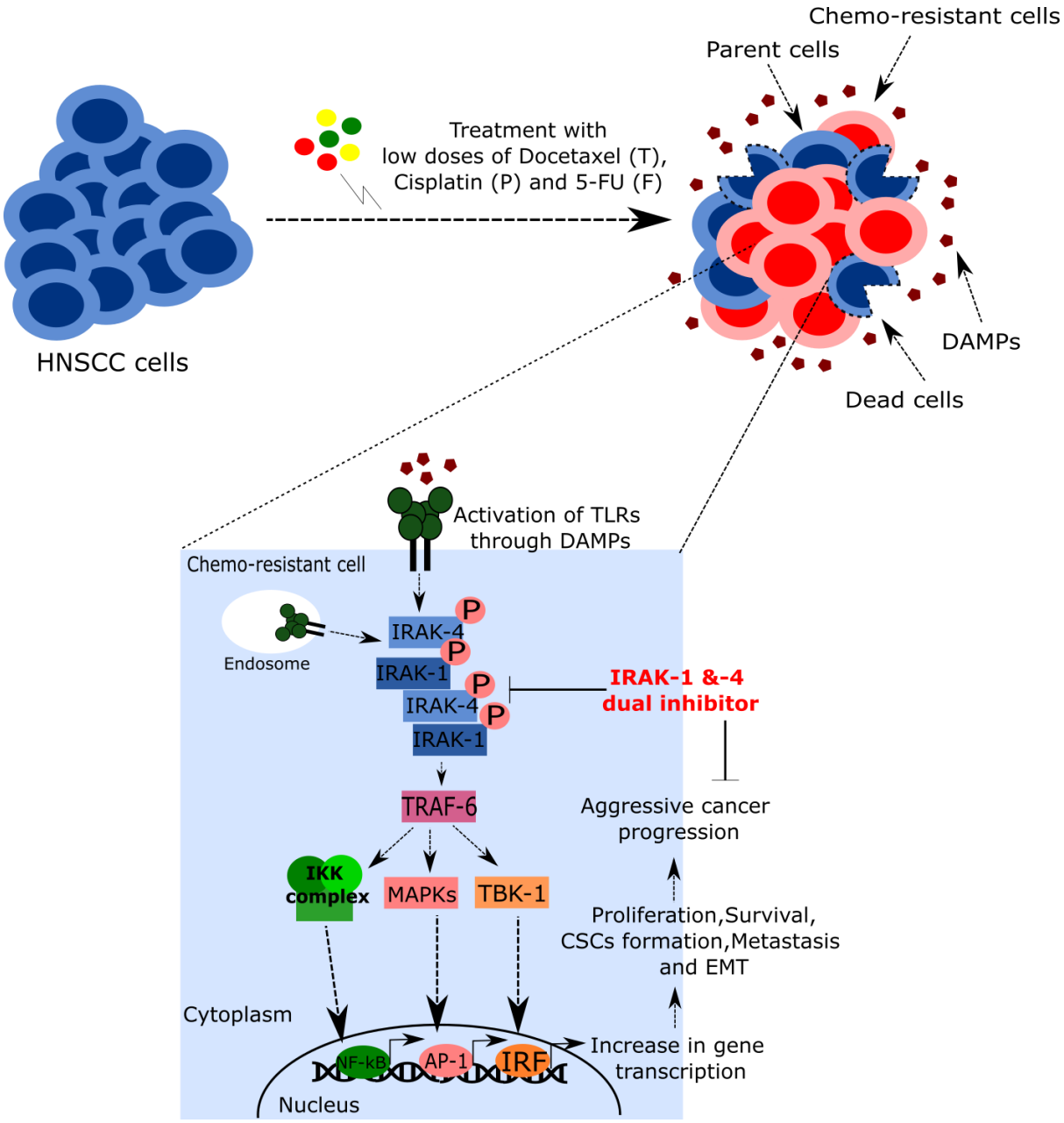
A cell line-based model to mimic chemo-resistance to TPF (combination of docetaxel, cisplatin and 5-fluorouracil) treatment was developed and validated using the same laryngeal origin HEp-2 HNSCC parent cell line. TPF chemo-resistant state demonstrated equivalent expression of TLRs comparable to parent line. Chemo-resistant state demonstrated over-

expression of both IRAK-1 and IRAK-4 along with increased phosphorylation as compared to the parent line. Data suggested presence of higher magnitude of TLR signaling in chemo-resistant state in HNSCC.

The chemo-resistant cell line exhibited enhanced proliferative potential, survival, stemness and metastatic capability as compared to the parent cell line. Pharmacological inhibition of TLR signaling through a small molecule based IRAK-1 &-4 dual inhibitor had a cytostatic effect on chemo-resistant cells along with suppressing most of the studied markers associated with pro-oncogenic effects. Data established the rationale to test TLR inhibitor as anti-cancer moiety in chemo-resistant HNSCC state as stand-alone as well as chemo-dose sparing combination therapy.

Adjuvant treatment of TLR inhibitor (IRAK-1 &-4 dual inhibitor) in combination with individual chemo-drugs docetaxel, cisplatin or 5-fluorouracil (5-FU) effectively suppressed the pro-oncogenic effects of the chemo-resistant cells while the effects were minimal to moderate in parent cells. Our study provides insights into the pro-oncogenic role of IRAK-1 &-4 mediated TLR signaling in HNSCC in native and the chemo-resistant state. TLR inhibitor was also found effective in reducing the impact of chronic chemo-drugs exposure towards aggressive cancer progression. This study showed amelioration of pro-oncogenic markers gained due to chemo-drug therapy alone. Study showed the potential use of IRAK based TLR inhibitor as chemo-drug dose sparing regimen. Such effects can not only delay the chemoresistance acquisition in patients but can also relive them from toxicities associated with chemo-drugs.

Our results provide proof of concept and establish rationale to test a novel therapy design for TPF-resistant HNSCC. This can ameliorate the effect of the drugs administered at minimal concentrations and suppress the toxic side effects mediated by them. Findings need to be validated in other HNSCC cell lines, pre-clinical animal model/s, and in clinical settings to establish TLR signaling through IRAKs as a biomarker and therapeutic target for TPF-resistant HNSCC.




**Graphical abstract**: **Role of TLR signaling in HNSCC Progression**