7. SUMMARY

This study establishes the role of TLR signaling in HNSCC progression in native and chemo-resistant state using laryngeal origin cell line HEp-2 as experimental system. Blocking the TLR signaling can be a potential therapeutic target for HNSCC. The key findings of the study are as follows:

- All the TLRs were found expressed in laryngeal origin HNSCC cell line HEp-2 except TLR 2 and TLR 10.
- TLR signaling was constitutively ON in HEp-2 as reflected by phosphorylated state of intermediate signaling kinase IRAK.
- Blocking TLR signaling pathway through downstream signaling kinases IRAK-1 and IRAK-4, using IRAK-1 &-4 dual inhibitor had impact on the growth of these cells with an IC_{50} of ~22µM.
- Chemo-resistant HEp-2 showed equivalent expression of all TLRs as parent HEp-2, along with the absence of TLR 2 and TLR 10. TLR signaling was constitutively active with an increased magnitude in chemo-resistant HEp-2 reflected by the over-expression and increased phosphorylation of IRAK-1 and -4 in resistant cells than in parent cells. IRAK-1 and IRAK-4, hence, may serve as potential clinically relevant biomarkers of TPF-resistance HNSCC.
- Chemo-resistant lineage had increased expression of markers of survival, proliferation, CSCs formation, metastasis and EMT with greater oncogenic potential.
- Blocking TLR signaling using IRAK-1 &-4 dual inhibitor suppressed the pro-oncogenic features of the chemo-resistant cells but not of parent cells, except moderate effect on the proliferation of parent cells.
- Chemo-drug exposure enhanced the pro-oncogenic attributes such as proliferation potential, stemness of HNSCC cells, in both cell lines. Inhibition of TLR signaling in this condition has a more compelling effect through suppression of this state in the resistant HNSCC cells compared to parent HNSCC cells.
- Our study provides a proof for concept of testing IRAK based TLR inhibitor for TPF-resistant HNSCC. Findings need to be confirmed in more than one cell line as well as in *in-vivo* pre-clinical and clinical settings.