CHAPTER 7

SUMMARY & CONCLUSION
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Our studies demonstrate the following-

- **Mcl-1 isoform expression in oral cancer**-
  - High expression of Mcl-1 transcripts / proteins in oral cancer cell lines, premalignant oral submucous fibrosis & oral tumors as compared to normal mucosa.
  - Five to ten fold higher expression of anti-apoptotic, full length Mcl-1L isoform versus the short & extra short Mcl-1S & Mcl-1ES isoforms in oral tumors & cell lines.
  - High ratio of Mcl-1L/Mcl-1S in oral tumors versus adjacent normals.
  - Significant correlation of high Mcl-1L expression with node positivity, advanced tumors and poor overall survival of oral cancer patients.

- **Thus, the predominantly expressed anti-apoptotic Mcl-1L isoform appears to be an independent prognostic factor for oral cancer.**

- **Mechanism of Mcl-1 over expression**-
  - Absence of genomic alterations in Mcl-1 gene (Promoter, 2 introns & 3 exons) in 10 oral cell lines.
  - Presence of 6-nt polymorphism in Mcl-1 promoter of SCC15 & SCC40 cancer cells & absence of 18-nt polymorphism in 11 Mcl-1 expressing oral cell lines.
  - Presence of 18-nt polymorphism in 7/20 (35%) healthy volunteers and 9/40 (23%) oral cancer patients.
  - Presence of 6/18-nt polymorphism did not correlate with either Mcl-1 gene expression or with clinico-pathological parameters of oral cancer patients.
  - Occurrence of two reported SNP’s namely C<A-324 &G<C-386 in Mcl-1 gene promoter of oral cancer patients.
  - Phosphorylation of Mcl-1 protein at Ser-159 & Thr-163 residues, which is essential for its detection by E3 ligases and further proteosomal degradation.

- **Genomic alterations, 6/18-nt polymorphism in Mcl-1 gene & altered phosphorylation of Mcl-1 protein are possibly not responsible for its overexpression in oral cancers.**
Role of Mcl-1 in Radioresistance and Chemoresistance-

- In relatively radioresistant AW8507 versus FBM, post IR-
  - Consistent & prolonged high expression of Mcl-1L mRNA & protein and decrease in percent of apoptosis.
  - Higher anti-apoptotic versus pro-apoptotic proteins ratios (Mcl-1L/Mcl-1S, Mcl-1L/Bax & Bcl-xl/Bax).
- High expression of Mcl-1 in the radioresistant sublines of AW13516 cell line obtained using fractionated irradiation.
- Combined treatment of Mcl-1L knockdown and IR resulted in increased nuclear condensation, upregulation of Bax & induction of apoptosis as compared to any treatments alone.
- Double knockdown of Mcl-1 & Bcl-xl in combination with Cisplatin significantly reduced cell viability & proliferation as compared to any treatment alone.
- Small molecule anti-Mcl-1 drug, Obatoclax in combination with Cisplatin exhibited significantly higher induction of cell death and reduction cell proliferation as compared to any treatment alone.

Mcl-1L downregulation potentially enhanced radiosensitivity and chemosensitivity of oral cancer cells in vitro.

Thus, our studies demonstrate overexpression of anti-apoptotic Mcl-1L isoform in oral cancers. Further Mcl-1L appears to be an important pro-survival and radioresistance/chemoresistance related factor, influencing outcome of oral cancer patients. It may therefore be a potential therapeutic target in oral cancers.