**Introduction**

- Sym004 is a novel strategy to target EGFR that is a mixture of two anti-EGFR antibodies directed against distinct epitopes on domain III of EGFR.
- Consistent with recent reports that showed a synergistic receptor down-regulation by combining noncompetitive anti-EGFR antibodies, Sym004 induces rapid and efficient internalization and degradation of EGFR.
- Sym004 exhibited pronounced tumor growth inhibition in a recent study (Cancer Res. 70:588–597, 2010).
- In the current study, we examine the capacity of Sym004 to augment radiation (XRT) response in non-small cell lung carcinoma (H226 & H292) and head and neck (H&N) squamous cell carcinoma (SCC-6 & SCC-1483) model systems.

**Sym004 induces EGFR degradation and inhibits proliferation**

(A) Western blots depict the effect of Sym004 (10 μg/ml) on EGFR protein expression.
(B) Effect of Sym004 on EGFR mRNA expression was determined by quantitated PCR 0–48 hrs following treatment.
(C) Effect of Sym004 on cellular proliferation was determined using CCK-8 proliferation assay.

**Sym004 regulates radiation-induced survival signaling, cell cycle progression and apoptosis**

(A) Western blots depict the effect of Sym004 on survival signaling 0–24 hrs after 6 Gy XRT.
(B) Flow cytometry depicts the effect of Sym004 on cell cycle progression 0–2 days after XRT.
(C) Effect of Sym004 on apoptosis was determined by Annexin-V/PI cytometry analysis 48 hrs after XRT. The percentage of cells in early apoptotic population (Annexin+/PI+) was shown in red and Sym004-induced apoptosis was highlighted by dotted circle.

**Sym004 augments radiation response in human tumor xenografts**

Effect of Sym004 on radiation response was examined in H226 and SCC-1483 tumor xenografts with fractionated or single dose of Sym004 and XRT.

(A) In H226, Sym004 was delivered via i.p., twice per week at a dose of either 1.6 (S1.6) or 4.8 (S4.8) mg/kg and XRT was delivered twice per week at a dose of 1.5 Gy from day 24 to day 48 (purple line) following tumor inoculation.
(B, Left) In SCC-1483, fractionated Sym004 (10 mg/kg) and XRT (3 Gy) were applied in the indicated time interval (purple line).
(B, Right) A single dose of Sym004 (80 mg/kg) and XRT (18 Gy) were applied at day 10.

**Conclusions**

- Sym004 exhibits significant antiproliferative effect and induces rapid EGFR degradation without inhibiting EGFR gene expression in various lung and H&N cancer cell lines (1).
- Sym004 enhances radiosensitivity in part by inhibiting key molecules involved in repairing radiation-induced double strand breaks. Sym004 significantly inhibits nuclear import of DNAPK (red dotted square in 2B)
- Sym004 inhibits radiation-induced survival signaling and cell cycle progression (3A, B). Sym004 also augments radiation-induced apoptosis (3C).
- Applying Sym004 to mice harboring H226 or SCC-1483 tumor xenografts, Sym004 exhibits profound antitumor effect when combined with either single or fractionated radiation (4).
- Our data indicates that Sym004 significantly augments radiation response in lung and H&N tumors. Sym004 represents a promising EGFR targeting agent reflecting distinct mechanism of action to trigger rapid and efficient down-regulation of EGFR.

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