SuppleSafety and Tumor-specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer

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Supplemental Material

Assessment of pharmacokinetics

Pharmacokinetic assessments were performed prior to infusion, 2hr after study drug, 24hr, 3–4 days, 15 days, and 30 days. Aliquots of plasma samples (2.5μL) were mixed with 4X sample buffer and resolved by NuPAGE 4–12% Bis-Tris gel (Invitrogen Corporation; Carlsbad, CA). A known amount of cetuximab-IRDye800 was assessed by gel electrophoresis and imaged at 800nm (Pearl Impulse) to verify cetuximab-IRDye800 at the 150kDa protein marker. For each cohort, total mg of cetuximab-IRDye800 was calculated at each time-point after quantification using Image Studio software (LI–COR Biosciences). Mean fluorescence intensity from size-matched ROI's was recorded and the mg of cetuximab-IRDye800 contained in each band was calculated based on mean counts from a set of cetuximab-IRDye800 standards. Total plasma mg of cetuximab-IRDye800 at each time point was determined based on patient dose and estimated total body blood weight. Total plasma mg values were averaged across patients for each cohort at every time point, and a scatter plot was generated to determine plasma clearance of cetuximab-IRDye800.

Supplemental Figure Legend

Supplemental Figure S1 Pharmacokinetics of cetuximab-IRDye800. **(A)** Total Plasma mg of cetuximab-IRDye800 over time for each cohort with standard deviations. **(B)** SDS-PAGE of plasma samples from patients within each cohort showing plasma cetuximab-IRDye800 (150kD) and free IRDye800 (1Kd).

Supplemental Figure S2 Fluorescent imaging of positive margin using intraoperative instrument. Pathologist-confirmed **(A)** positive and **(C-E)** negative margins are shown from patient 9 (62.5mg/m^2 dose). **(B)** Relative fluorescent units of positive and negative margins was calculated. Data are relative fluorescent units ± SD. Pathologist confirmed presence of tumor using adjacent H&E stained sections of respective margins. Image scaling was kept consistent for fluorescence and brightfield during image processing.

Supplementary Figure S3 Opportunity for tissue saving procedures. **(A)** Brightfield image of cutaneous squamous cell carcinoma from cohort 1 (2.5mg/m^2 dose) with surgeon margins outlined in the temple region prior to resection. **(B)** Ex vivo brightfield image of resected primary tumor with **(C)** respective closed-field fluorescence and **(D)** wide-field fluorescence images.