IRDye800CW

IRDye 800CW near-infrared fluorescent dyes can be used for protein and antibody labeling, or nucleic acid applications with high labeling density.

These 800 nm channel near-infrared dyes are superior for Western blotting and immunocytochemical assays, including In-Cell Western™ assays and On-Cell Western cell-based assays, as well as for protein arrays, microscopy, tissue section imaging, and in vivo imaging and optical probe development.

IRDye 800CW dye is characterized by high water solubility and salt tolerance, low non-specific binding to cellular components, and a high signal-to-noise ratio.

IRDye 800CW dye-conjugated agents and probes are currently propelling more than a dozen Phase I or Phase II clinical trials, more than any other near-infrared fluorescent dye.

TIM3 monoclonal antibody was conjugated to a near-infrared fluorescent dye, IRDye-800CW (800CW). The TIM3 experimental conjugate and isotype control were assessed for specificity with immunofluorescent staining and flow cytometry in murine cell lines (GL261 glioma and RAW264.7 macrophages). C57BL/6 mice with orthotopically implanted GL261 cells were imaged in vivo over 4 days after intravenous TIM3-800CW injection to assess tumor-specific uptake. Cell-specific uptake was then assessed on histologic sections.

The experimental TIM3-800CW, but not its isotype control, bound to RAW264.7 macrophages in vitro. Specificity to RAW264.7 macrophages and not GL261 tumor cells was quantitatively confirmed with the corresponding clone of TIM3 on flow cytometry. In vivo fluorescence imaging of the 800CW signal was localized to the intracranial tumor and significantly higher for the TIM3-800CW cohort, relative to non-targeting isotype control, immediately after tail vein injection and for up to 48 h after injection. Resected organs of tumor-bearing mice showed significantly higher uptake in the liver and spleen. TIM3-800CW was seen to co-stain with CD3 (13%), CD11b (29%), and CD206 (26%).

They propose fluorescent imaging of immune cell imaging as a potential strategy for monitoring and localizing immunologically relevant foci in the setting of brain tumors. Alternative markers and target validation will further clarify the temporal relationship of immunosuppressive effector cells throughout glioma resistance. [1].

Kurbegovic et al. developed and evaluated a new uPAR-targeted optical probe, IRDye800CW-AE344, for fluorescence guided surgery (FGS). Methods: In the present study we characterized the fluorescent probe with regard to binding affinity, optical properties, and plasma stability. Further, in vivo imaging characterization was performed in nude mice with orthotopic human patient derived glioblastoma xenografts, and we performed head-to-head comparison within FGS between our probe and the traditional procedure using 5-ALA. Finally, the blood-brain barrier (BBB) penetration was characterized in a 3D BBB spheroid model. Results: The probe effectively visualized Glioblastoma in vivo with a tumor-to-background ratio (TBR) above 4.5 between 1 to 12 h post injection and could be used for FGS of orthotopic human glioblastoma xenografts in mice where it was superior to 5-ALA. The probe showed a favorable safety profile with no evidence of any acute toxicity. Finally, the 3D BBB model

showed uptake of the probe into the spheroids indicating that the probe crosses the BBB. Conclusion: IRDye800CW-AE344 is a promising uPAR-targeted optical probe for FGS and a candidate for translation into human use. [2].

Binding affinity of 800CW-TATE was evaluated using [177Lu] Lu-DOTA-Tyr3-octreotate displacement assays. Tumor uptake was determined by injecting 800CW-TATE in (SSTR2-positive) NCI-H69 or (SSTR2-negative) CH-157MN xenograft bearing mice and FMT2500 imaging. SSTR2-specific binding was measured by comparing tumor uptake in NCI-H69 and CH-157MN xenografts, blocking experiments and non-targeted IRDye800CW-carboxylate binding. Tracer distribution was analyzed ex vivo, and the tumor-to-background ratio (TBR) was calculated. SSTR2 expression was determined by immunohistochemistry (IHC). Lastly, 800CW-TATE was incubated on frozen and fresh meningioma specimens and analyzed by microscopy.

Results: 800CW-TATE binding affinity assays showed an IC50 value of 72 nM. NCI-H69 xenografted mice showed a TBR of 21.1. 800CW-TATE detection was reduced after co-administration of non-fluorescent DOTA-Tyr3-octreotate or administration of IRDye800CW. CH-157MN had no tumor specific tracer staining due to absence of SSTR2 expression, thereby serving as a negative control. The tracer bound specifically to SSTR2-positive meningioma tissues representing all WHO grades.

Conclusion: 800CW-TATE demonstrated sufficient binding affinity, specific SSTR2-mediated tumor uptake, a favorable biodistribution, and high TBR. These features make this tracer very promising for use in MFGS and could potentially aid in safer and a more complete meningioma resection, especially in high-grade meningiomas or those at complex anatomical localizations. [3] [4]

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