Comparative Biodistribution and Radiotherapeutic Efficacy of the Fibroblast Activation Protein (FAP)–Targeting Agents FAP-2286 and FAPI-46

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ABSTRACT

FAP-2286 Showed Durable Cellular Retention Compared to FAPI-46 in HEK-FAP cells

INTRODUCTION

• Fibroblast activation protein (FAP) is a membrane-bound protease under investigation as a pan-cancer imaging and therapeutic target given its limited expression in normal adult tissues but high expression on cancer-associated fibroblasts.

• FAP-targeting agents, FAP-2286 and FAPI-46, have shown great promise as positron emission tomography (PET) imaging agents when covalently linked to Gallium-68 (68Ga).

• FAP-2286 is a quinoline-based, FAP-targeting, small-molecule tetraazacyclododecanetetraacetic acid (DOTA) conjugate and has been shown to yield high tumor-to-background ratios in patients with various cancers.

• FAP-2286, a FAP-binding peptide monoclonal covalently linked to the radionuclide chelator DOTA, constitutes a new class of FAP-targeting modalities. PET scintigraphy of 68Ga-FAP-2286 demonstrated high uptake in neoplastic tissues and long retention in normal tissues of patients with solid cancers.

The goals of these studies were to evaluate the biodistribution of FAP-2286 and FAPI-46 when covalently linked to radionuclides and to correlate these results with the efficacy observed in the HEK293 FAP-overexpressing tumor model.

METHODS

• Biochemical and cellular assays: The binding kinetics \( K_a \) of the test compounds to amyloid-precursor-like-family protein was determined by surface plasmon resonance. Human recombinant FAP was incubated with test compounds. Median fluorescence intensity was measured by flow cytometry.

• In vivo biodistribution and efficacy studies: Female NMRI nude mice were subcutaneously implanted with 2×10^6 HEK-FAP cells (n=10 per group) and used to evaluate the biodistribution of 68Ga-FAP-2286 and 177Lu-FAP-2286. 68Ga-FAP-2286 and 177Lu-FAP-2286 single dose was administered. Studies were performed on an Excelsior PET/CT scanner (Kitronix, Chiswick, UK).

• Internalization assay: AlexaFluor488-labeled FAP-2286 and FAPI-46 were internalized into HEK-FAP cells and retained intracellular signal with minimal cell damage.

• PET/CT Imaging of both FAP-2286 and FAPI-46 was performed to assess tumor uptake and retention in vivo.

RESULTS

In Vivo Biodistribution of 68Ga-FAP-2286 and 177Lu-FAP-2286 in HEK-FAP Tumor-Bearing Mice by PET/CT Imaging

- In vivo with 68Ga-FAP-2286 and 177Lu-FAP-2286 showed similar tumor uptake with 9.8 ± 0.7 and 9.3 ± 1.8 %ID/g at 30 minutes post injection (p=0.945). FAP-2286 demonstrated high tumor uptake in neoplastic tissues and long retention in normal tissues of patients with solid cancers.

- The prolonged tumor retention of FAP-2286 correlated with a higher intracellular retention of the therapeutic (177Lu-FAP-2286) and imaging (68Ga-FAP-2286) agent in multiple tumor xenografts.

- FAP-2286 and FAPI-46 showed similar extracellular binding to HEK-FAP cells that was completely blocked by unlabeled competitor.

- FAP-2286 showed greater cell surface and intracellular fluorescence compared to FAPI-46 starting at 8 hours of incubation, and only 72 hours, FAP-2286 was not detected within FAP-2286-retained intracellular signal with minimal cell surface fluorescence.

- FAP-2286 showed durable cellular retention compared to FAPI-46 in HEK-FAP cells.

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