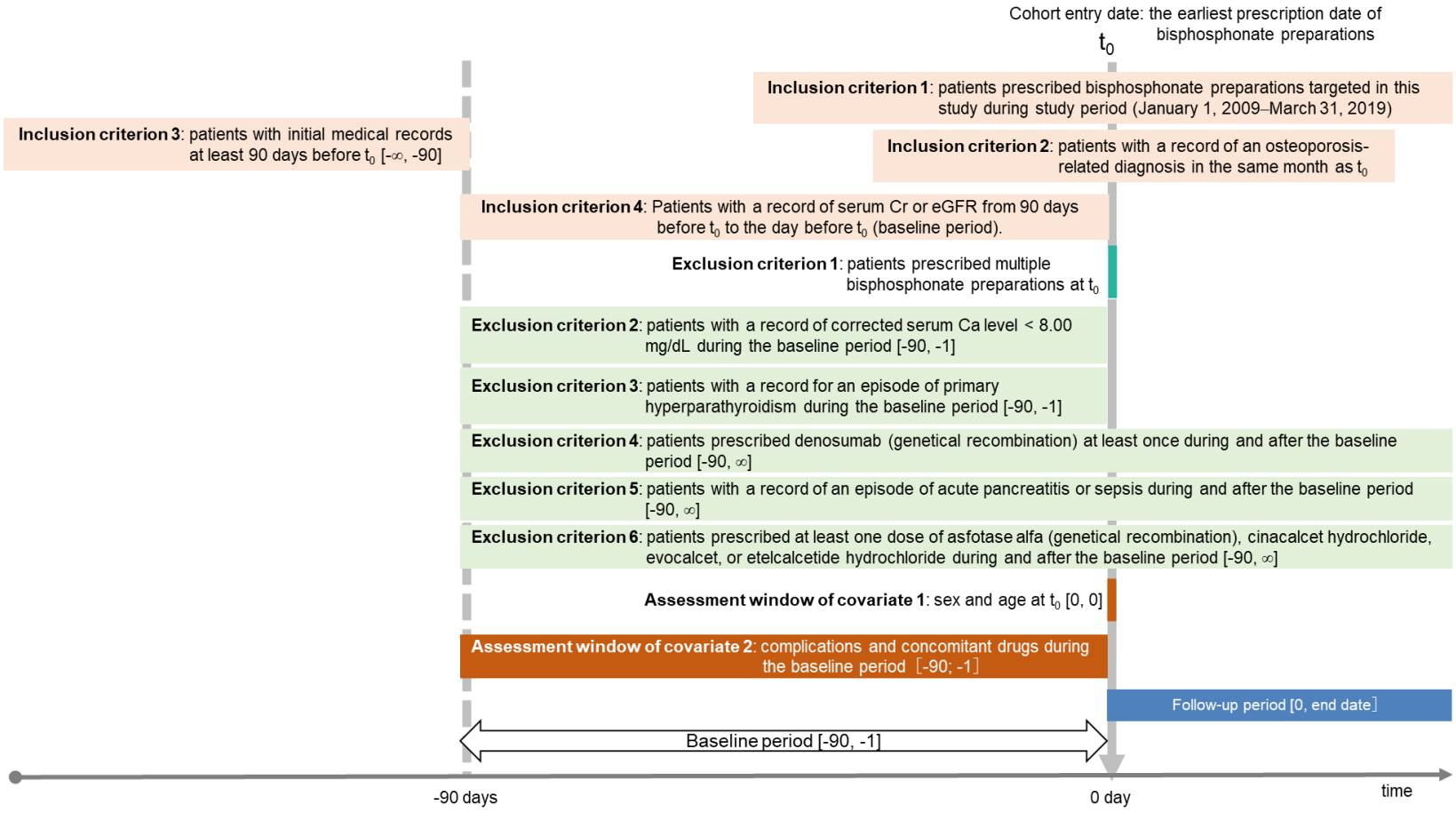Additional file 1: Figure S1 Design diagram