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OPEN

A mAb for the detection of the antiretroviral drug emtricitabine

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Antibody-based testing for emtricitabine (FTC), a critical component of pre-exposure prophylaxis and antiretroviral therapy, would provide low-cost detection for clinical monitoring to improve adherence. We developed a mAb (5D2) to FTC and demonstrated its high specificity and physiologically relevant linear range of detection in a competitive enzyme immunoassay. Thus, this mAb is a key reagent that will enable simple and low-cost lateral flow assays and enzyme immunoassays for adherence monitoring.

Antiretroviral therapy (ART) and pre-exposure prophylaxis (PrEP) are the cornerstones of HIV prevention and elimination strategies and are very effective when taken consistently [1]. Antiretroviral regimens containing the nucleoside reverse transcriptase inhibitor emtricitabine (FTC) are widely used in first-line therapy of HIV-infected persons. Daily PrEP with the combination of FTC and tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) is also recommended for HIV prevention in populations at risk for infection [2]. Inadequate adherence to daily ART and PrEP has been shown to reduce effectiveness and public health benefit [3–7]. Pharmacologic monitoring of adherence to these regimens provides an objective measurement of compliance and has been shown to predict the efficacy of PrEP [4,8]. Current methods to measure drugs rely on complex analytical tests using liquid chromatography and tandem mass spectrometry that are costly, require skilled personnel, and are not implementable at point-of-care (POC) testing sites [9–11]. Furthermore, due to limitations in self-reporting and pill-counting methods of adherence monitoring [12,13], a direct method for measuring FTC levels provides an objective marker that could have broad implications on the ability of care providers to monitor and counsel adherence to ART and PrEP [14]. Thus, there is a need to develop a simple, scalable, and inexpensive assay to detect antiretroviral drugs for adherence monitoring in people on PrEP and ART. Antibody-based tests, such as lateral flow diagnostic strips, allow for the development of POC assays that can provide real-time feedback to patients to improve adherence [14]. Several groups have developed immunodiagnostic tests for tenofovir detection demonstrating the validity of this approach on small antiretroviral drugs [15–17]. However, tenofovir concentrations vary substantially in persons treated with either TDF or TAF requiring different assay modifications and varied reference cutoffs [16,18,19]. Because FTC dosage is the same in ART and PrEP regimens, we posit that an antibody-based FTC assay will provide a universally simple tool for adherence monitoring of ART and PrEP. Thus, we developed an FTC-specific antibody to facilitate the development of these critical tools.

Three BALB/C mice were immunized with FTC conjugated to an extremely immunogenic carrier molecule, the *Limulus polyphemus* hemocyanin II. After 5 months, serologic responses to FTC were assayed to determine the magnitude and specificity of the responses for FTC. Twenty-nine hybridomas were generated from the mouse with the best anti-FTC responses, expanded

for 3 weeks, and screened for reactivity and specificity to FTC. Twelve antibodies were chosen for further characterization.

Due to FTC's small size (247.2 Da) relative to antibody-binding surfaces, quantitation by enzyme immunoassay (EIA) using a single antibody can only be performed in a competitive format where known concentrations of free FTC would compete with an immobilized FTC-substrate for antibody binding. Only one antibody (5D2) exhibited specificity for FTC (Fig. 1a) in this assay. The dynamic linear range of the purified antibody for FTC detection was from 100 μg/ml to 3 μg/ml (Fig. 1b). The limit of detection for this assay was estimated by comparing the number of wells at 1.56 and 3.125 μg/ml that had greater than 0.1 difference in absorbance from the paired no FTC condition. All 10 of the 3.125 μg/ml wells were distinguishable from no FTC (Fig. 1c) while only seven of 10 of the 1.56 μg/ml wells were distinguishable from no FTC (Fig. 1d). Thus, the limit of detection of our FTC EIA is estimated to be 3 μg/ml.

Because FTC is a nucleoside analog, the mAb 5D2 was tested for cross-reactivity to nucleosides and FTC structural analogs and anabolites. A competitive EIA was used to evaluate the reactivity of 5D2 to naturally occurring structural analogs consisting of nitrogenous nucleic acid bases, ribonucleosides, and deoxyribonucleosides. 5D2 did not bind to any of the 10 ribonucleosides or deoxyribonucleosides tested, nor did it bind to FTC-triphosphate, the pharmacologically active anabolite of FTC (Fig. 1e). Assessment of two structurally similar antiretroviral drugs, lamivudine (3TC) and abacavir, along with the pharmacological intermediate, FTC-diphosphate, further demonstrated the high specificity of 5D2 binding to FTC (Fig. 1f). FTC is an analog of deoxycytidine with two significant differences: first, a sulfur substitution for the 3′ carbon, a motif that it shares with 3TC, and second, a fluorine addition to the pyrimidine ring which is unique to FTC. Given that 5D2 does not recognize cytidine, deoxycytidine, nor 3TC, we hypothesize that the fluoride-modified pyrimidine ring is the primary antigenic motif recognized by 5D2. Indeed, fluorine substitutions, which are widely used in biomedical drug development [20], have been shown to increase antibody affinity when employed in cocaine-hapten and anti-cancer sialyl-Tn vaccine studies [21,22]. However, despite strong affinity for the native FTC structure, this antibody does not bind to the di-phosphate or tri-phosphate anabolites. It is possible that the phosphate groups could first, mask the antigenic contribution of the 5′ hydroxyl; second, sterically hinder 5D2 from binding to

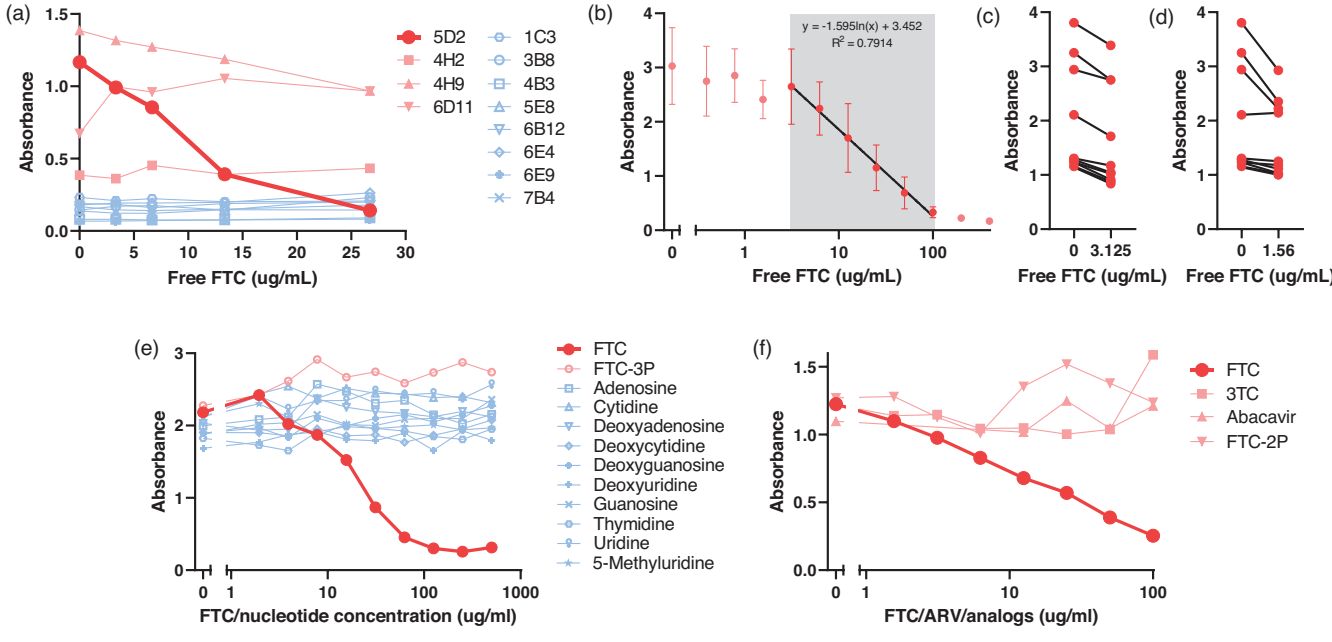

Fig. 1. Competitive enzyme immunoassay characterization of anti-FTC antibodies. (a) Twelve hybridoma supernatants were screened in a competitive enzyme immunoassay for specific binding to FTC. Reduction in binding to plate-bound FTC conjugates by increasing amounts of soluble FTC was observed with only one clone supernatant, 5D2 (red). High binders and low binders were indicated in pink or light blue, respectively. (b) The linear range of the FTC competitive enzyme immunoassay was determined with four replicate experiments. The gray box indicates the linear range (100–3.125 μg/ml). (c, d) The limit of detection was estimated by comparison of no FTC conditions to paired (c) 3.125 μg/ml and (d) 1.56 μg/ml wells. Ten samples across several experiments are displayed. (e, f) The specificity of 5D2 for FTC (red) was determined by competitive enzyme immunoassay where FTC was replaced with nucleosides (light blue) and FTC anabolites and drug analogs and anabolites (pink).

the putative fluoro-pyrimidine antigen; or third, modify the fluoro-pyrimidine motif's chemical properties (e.g., hydrophobicity, electrochemical charge, etc.). Further studies are needed to determine the exact immunological contacts between 5D2 and FTC.

Taken together, the present data support the utility of 5D2 in antibody-based assays developed for clinical adherence monitoring, including POC test formats that allow immediate and low-cost detection in clinical settings. Because daily dosing is known to concentrate FTC in the urine to high levels, ranging between 10 and 100 μg/ml [23], our data point to the feasibility of 5D2 use in POC lateral flow assays for urine testing which has additional feasibility and acceptability advantages [24]. For ART, a simple urine test for FTC may allow for enhanced adherence counseling and might better inform the need for viral load and drug resistance testing. In sum, despite the challenges of raising antibodies to a small nucleoside analog, we were able to generate a highly specific antibody to FTC. The availability of 5D2 will enable the development of low-cost antibody-based adherence tests for FTC to improve the effectiveness of PrEP and ART.

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Conflicts of interest

A.S.Y, T.C.G., C-P.P., J.P., W.M.S., and W.H. are named in US Government patents (US20210253738) and patent applications on 'Monoclonal antibody for the detection of the antiretroviral drug emtricitabine (FTC, 2′,3′-DIDEOXY-5-FLUORO-3′-THIACYTIDINE)'.

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