Development of a First-in-Class Oral Selective ER Covalent Antagonist (SERCA) for the Treatment of ERαWT and ERαMUT Breast Cancer

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Introduction: Mutations in estrogen receptor alpha (ERα) - detected in up to 30% of patients who have relapsed during endocrine treatments—are constitutively activating and promote partial resistance to endocrine therapies. Since a significant proportion of therapy-resistant breast cancer metastases continue to be dependent on ER signaling, there remains a critical need to develop the next generation of ERα antagonists that can overcome aberrant ERα activity. We identified H3B-6545 as a member of a novel class of ERα antagonists referred to as galenic ER Covalent Antagonists (SERCA), which inactivates both wild-type and mutant ERα by targeting C300 and enforcing a unique antigenic conformation. Here we describe the in vitro and in vivo properties of H3B-6545.

H3B-6545 is a first-in-class Selective ER Covalent Antagonist (SERCA)

Figure 1: ERα antagonists exhibit variable partial resistance to standard of care (SoC) anti-estrogen therapies. A, Western blot showing ERα expression in various engineered MCF7 breast cancer cell lines. B, Dose response curve for ATP-based cell viability assay following a 6 day treatment with fulvestrant (left) or 4-OHT (right).

H3B-6545 promotes a unique ERα conformation and imparts divergent biology

Figure 2: H3B-6545 covalently engages C300 of ERα and is non-degradable. A, Sequence alignment highlighting cysteine at C300 position. B, Mass spectrometry-based validation of covalent engagement of H3B-6545 with ERαWT. C, CD205 binding assay performed in MCF7 cells with indicated concentrations of fulvestrant, H3B-6545, and abemaciclib with conformation without degrading probes. Luciferase signal was measured after 24 hours of compound treatment at 10 μM. Red = strong recruitment, blue = weak recruitment, and white = average recruitment. D, Fold change in IP-MS result in the linked endothelial carcinoma cell line following a 24 hour treatment with the indicated compounds.

H3B-6545 inhibits ERα activity and growth of ERαWT-positive breast cancer cell lines

Figure 3: H3B-6545 potently inhibits ERα activity and proliferation of ERαWT-positive breast cancer cell lines. A, Dose-dependent inhibition in MCF7 cells mediated through overexpression of ERαWT, ERαMUT, palbociclib, and abemaciclib with conformation without degrading probes. B, Table summarizing G1/G0 values from ATP-based cell viability assays. MCF7, HCC1215, BT474, and T47D1 are cell lines engineered to express ERαWT or ERαMUT. ERαWT in the mutant breast cancer cell line. NA, 50% inhibition not achieved.

H3B-6545 demonstrates efficacy in ERαWT and ERαMUT PDX models

Figure 4: H3B-6545 potently inhibits ERα activity and suppresses proliferation of ERαWT-positive breast cancer cell lines. A, Dose-dependent inhibition in MCF7 cells mediated through overexpression of ERαWT, ERαMUT, palbociclib, and abemaciclib with conformation without degrading probes. B, Table summarizing G1/G0 values from ATP-based cell viability assays. MCF7, HCC1215, BT474, and T47D1 are cell lines engineered to express ERαWT or ERαMUT. ERαWT in the mutant breast cancer cell line. NA, 50% inhibition not achieved.

Summary

• H3B-6545 is a first-in-class selective, covalent antagonist that binds ERα irreversibly, and enforces a novel antagonist conformation without degrading ERα
• H3B-6545 potently suppresses ERαWT pathway activity and growth of ERαWT-positive breast cancer lines in vitro and in vivo
• H3B-6545 in combination with palbociclib enhances efficacy in ERαWT PDX model
• H3B-6545 is currently being evaluated in an ongoing Phase 1/2 study in women with locally advanced or metastatic ERα+/PR+ breast cancer (NCT03325976)

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